

FOR *IN VITRO* DIAGNOSTIC USE.

cobas[®] DNA Sample Preparation Kit

DNA SP

24 Tests

P/N: 05985536190

cobas[®] EGFR Mutation Test

EGFR

24 Tests

P/N: 06471463190

NOTICE: The purchase of this product allows the purchaser to use it for amplification and detection of nucleic acid sequences by polymerase chain reaction (PCR) and related processes for human *in vitro* diagnostics. No general patent or other license of any kind other than this specific right of use from purchase is granted hereby.

TABLE OF CONTENTS

INTENDED USE	2
SUMMARY AND EXPLANATION OF THE TEST	2
PRINCIPLES OF THE PROCEDURE	3
Specimen Preparation	3
PCR Amplification	3
Target Selection	3
Target Amplification	3
Automated Real-time Mutation Detection	3
Selective Amplification	3
REAGENTS	4
WARNINGS AND PRECAUTIONS	6
STORAGE AND HANDLING REQUIREMENTS	6
MATERIALS PROVIDED	7
MATERIALS REQUIRED BUT NOT PROVIDED	7
Instrumentation and Software	8
SPECIMEN COLLECTION, TRANSPORT, AND STORAGE	8
INSTRUCTIONS FOR USE	8
Run Size	8
Workflow	8
Reagent Preparation	9
Deparaffinization of FFPET Sections Mounted on Slides	9
Deparaffinization of FFPET Sections not Mounted on Slides	9
SPECIMEN PREPARATION	10
DNA Isolation Procedure	10
DNA Quantitation:	11
Instrument Set-Up:	11
Test Order Set-up:	11
Dilution Calculation of Specimen DNA Stock:	11
Specimen Dilution	12
Preparation of Working Master Mixes (MMX-1, MMX-2 and MMX-3)	12
Preparation of Plate	13
Starting PCR	14
INTERPRETATION OF RESULTS	14
Retesting of Specimens with Invalid Results	14
QUALITY CONTROL	14

The Document Revision Information section is located at the end of this document.

Mutant Control	14
Negative Control	14
PROCEDURAL PRECAUTIONS.....	14
PROCEDURAL LIMITATIONS.....	14
NON-CLINICAL PERFORMANCE EVALUATION.....	15
Analytical Sensitivity – Limit of Blank.....	15
Analytical Sensitivity Using FFPET Specimen Blends.....	15
Minimal Tumor Content.....	16
Cross-Reactivity to Other Exon 19 and Exon 21 Mutations.....	17
Plasmid Samples.....	17
Specificity – Microorganisms and EGFR Homologs.....	18
Lung-related Microorganisms.....	18
Plasmids of EGFR Homologs.....	18
Interference.....	18
Necrotic Tissue.....	19
Repeatability.....	19
Specimen Handling Reproducibility.....	19
CLINICAL PERFORMANCE EVALUATION.....	19
Clinical Reproducibility.....	19
Correlation to Reference Method using Phase 3 Samples.....	20
Clinical Outcome Data.....	21
REFERENCES.....	22

INTENDED USE

The **cobas**[®] EGFR Mutation Test is a real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPET) human non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used as an aid in selecting patients with NSCLC for whom Tarceva[®] (erlotinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated.

Specimens are processed using the **cobas**[®] DNA Sample Preparation Kit for manual sample preparation and the **cobas z 480** analyzer for automated amplification and detection.

SUMMARY AND EXPLANATION OF THE TEST

Activating mutations in the gene encoding EGFR occur primarily in NSCLC, and result in constitutive activation of the kinase activity of the EGFR protein, thereby contributing to the oncogenic process.¹ The prevalence of these mutations in unselected cases of NSCLC is approximately 10% - 30%.^{2,3} However, these mutations occur more frequently, but not exclusively, in non-smoking/light-smoking female patients of Asian ancestry with adenocarcinoma histologies.⁴

The most common EGFR mutations in NSCLC include a variety of deletions in exon 19 and the substitution mutation L858R in exon 21; these mutations collectively constitute approximately 85% of EGFR mutations observed in NSCLC.⁵ The **cobas**[®] EGFR Mutation Test is a real-time PCR assay designed to detect deletion mutations in exon 19 and the substitution mutation L858R in exon 21. The **cobas**[®] EGFR Mutation Test is used as a companion diagnostic test for Tarceva[®], a compound that reversibly inhibits the kinase activity of EGFR, preventing autophosphorylation of tyrosine residues associated with the receptor and thereby inhibiting further downstream signaling that promotes cell survival and proliferation. Erlotinib binding affinity for EGFR exon 19 deletion or exon 21 L858R mutations is higher than its affinity for the wild-type receptor.⁶ Clinical trials have shown that patients with advanced NSCLC and with exon 19 deletion mutations or L858R substitution mutation in exon 21 that were treated with Tarceva[®] as first-line treatment, are likely to experience clinical benefit compared to patients treated with chemotherapy.^{3,7}

The **cobas**[®] EGFR Mutation Test detects the following deletion mutations in exon 19 of the EGFR gene: 2235_2249del15, 2236_2250del15, 2238_2252del15, 2239_2248>C, 2240_2254del15, 2240_2257del18, 2237_2253>TTGCT, 2237_2255>T, 2239_2256del18, and 2239_2257>GT, as well as the L858R substitution mutation 2573 T>G in exon 21 of the EGFR gene. All mutations are detected at 5% sensitivity with the exception of the 2240_2257del18 exon 19 deletion mutation, which is detected at a sensitivity of >10% (Table 3).

PRINCIPLES OF THE PROCEDURE

The **cobas**[®] EGFR Mutation Test is based on two major processes: (1) manual specimen preparation to obtain genomic DNA from FFPET; and (2) PCR amplification and detection of target DNA using complementary primer pairs and oligonucleotide probes labeled with fluorescent dyes. The test is designed to detect deletions and complex mutations in exon 19 and L858R in exon 21.

Mutation detection is achieved through PCR analysis with the **cobas z 480** analyzer. A mutant control and negative control are included in each run to confirm the validity of the run.

Specimen Preparation

FFPET specimens are processed and genomic DNA isolated using the **cobas**[®] DNA Sample Preparation Kit, a manual specimen preparation based on nucleic acid binding to glass fibers. A deparaffinized 5 µm section of an FFPET specimen is lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The amount of genomic DNA is spectrophotometrically determined and adjusted to a fixed concentration to be added to the amplification/detection mixture. The target DNA is then amplified and detected on the **cobas z 480** analyzer using the amplification and detection reagents provided in the **cobas**[®] EGFR Mutation Test kit.

PCR Amplification

Target Selection

The **cobas**[®] EGFR Mutation Test kit uses primers that define specific base-pair sequences for each of the targeted mutations. For the exon 19 deletion mutations, base pair sequences that range from 125 to 141 are targeted and; for the L858R substitution mutation in exon 21, a 138 base pair sequence is targeted; for the internal control in exon 28, a 87 base pair sequence is targeted. Amplification occurs only in the regions of the EGFR gene between the primers; the entire EGFR gene is not amplified.

Target Amplification

A derivative of *Thermus* species Z05-AS1 DNA polymerase is utilized for target amplification. First, the PCR reaction mixture is heated to denature the genomic DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The Z05 DNA polymerase, in the presence of divalent metal ion and excess dNTP, extends each annealed primer, thus synthesizing a second DNA strand. This completes the first cycle of PCR, yielding a double-stranded DNA copy which includes the targeted base-pair regions of the EGFR gene. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of amplicon DNA.

Automated Real-time Mutation Detection

The **cobas**[®] EGFR Mutation Test utilizes real-time PCR technology. Each target-specific, oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher molecule that absorbs (quenches) fluorescent emissions from the reporter dye within an intact probe. During each cycle of amplification, probe complementary to the single-stranded DNA sequence in the amplicon binds and is subsequently cleaved by the 5' to 3' nuclease activity of the Z05-AS1 DNA Polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, fluorescence of a characteristic wavelength can be measured when the reporter dye is excited by the appropriate spectrum of light. Three different reporter dyes are used to label the mutations targeted by the test. Amplification of the targeted EGFR sequences are detected independently across three reactions by measuring fluorescence at the three characteristic wavelengths in dedicated optical channels.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**[®] EGFR Mutation Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP).⁸ The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA but is always present in amplicon due to the use of dUTP in place of deoxythymidine triphosphate as one of the nucleotide triphosphates in the Master Mix reagents; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. The AmpErase enzyme, which is included in the Master Mix reagents, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at alkaline pH, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon.

REAGENTS**cobas® DNA Sample Preparation Kit**

(P/N: 05985536190)

DNA SP**24 Tests****DNA TLB**

(DNA Tissue Lysis Buffer)

Tris-HCl buffer
 Potassium chloride
 0.04% EDTA
 0.1% Triton X-100
 0.09% Sodium azide

1 x 10 mL

PK

(Proteinase K)

Proteinase K (lyophilized)

Xn  Proteinase K

Harmful

1 x 100 mg

DNA PBB

(DNA Paraffin Binding Buffer)

Tris-HCl buffer
 49.6% Guanidine hydrochloride
 0.05% Urea
 17.3% Triton X-100

Xn  49.6% (w/w) Guanidine HCl

Harmful

1 x 10 mL

WB I

(DNA Wash Buffer I)

Tris-HCl buffer
 64% Guanidine hydrochloride

Xn  64% (w/w) Guanidine HCl

Harmful

1 x 25 mL

WB II

(DNA Wash Buffer II)

Tris-HCl buffer
 Sodium chloride

1 x 12.5 mL

DNA EB

(DNA Elution Buffer)

Tris-HCl buffer
 0.09% Sodium azide

1 x 6 mL

FT

(Filter tubes with caps)

1 x 25 pcs

CT

(Collection Tubes)

3 x 25 pcs

cobas® EGFR Mutation Test

(P/N: 06471463190)

EGFR**24 Tests****EGFR MMX-1**

(EGFR Master Mix 1)

Tris buffer
 Potassium chloride
 Glycerol
 EDTA
 Tween 20
 3.13% Dimethyl sulfoxide
 0.09% Sodium azide
 <0.10% dNTPs
 <0.01% Z05-AS1 DNA polymerase (microbial)
 <0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)
 <0.01% Aptamer
 <0.01% Upstream and downstream EGFR primers
 <0.01% Fluorescent labeled EGFR probes

2 x 0.48 mL

EGFR MMX-2 (EGFR Master Mix 2)	2 x 0.48 mL
Tris buffer	
Potassium chloride	
Glycerol	
EDTA	
Tween 20	
3.13% Dimethyl sulfoxide	
0.09% Sodium azide	
<0.10% dNTPs	
<0.01% Z05-AS1 DNA polymerase (microbial)	
<0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)	
<0.01% Aptamer	
<0.01% Upstream and downstream EGFR primers	
<0.01% Fluorescent labeled EGFR probes	
EGFR MMX-3 (EGFR Master Mix 3)	2 x 0.48 mL
Tris buffer	
Potassium chloride	
Glycerol	
EDTA	
Tween 20	
3.13% Dimethyl sulfoxide	
0.09% Sodium azide	
<0.10% dNTPs	
<0.01% Z05-AS1 DNA polymerase (microbial)	
<0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial)	
<0.01% Aptamer	
<0.01% Upstream and downstream EGFR primers	
<0.01% Fluorescent labeled EGFR probes	
MGAC (Magnesium acetate)	6 x 0.2 mL
Magnesium acetate	
0.09% Sodium azide	
EGFR MC (EGFR Mutant Control)	6 x 0.1 mL
Tris buffer	
EDTA	
Poly-rA RNA (synthetic)	
0.05% Sodium azide	
<0.1% Plasmid DNA containing targeted EGFR sequences (microbial)	
<0.1% EGFR wild-type DNA (cell culture)	
DNA SD (DNA Specimen Diluent)	2 x 3.5 mL
Tris-HCl buffer	
0.09% Sodium azide	

WARNINGS AND PRECAUTIONS

A. **FOR *IN VITRO* DIAGNOSTIC USE.**

- B. This test is for use with FFPET NSCLC specimens.
- C. Do not pipette by mouth.
- D. Do not eat, drink or smoke in laboratory work areas.
- E. Avoid microbial and DNA contamination of reagents.
- F. Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- G. Do not use kits after their expiration dates.
- H. Do not pool reagents from different kits or lots.
- I. Gloves must be worn and must be changed between handling specimens and reagents to prevent contamination.
- J. To avoid contamination of the working Master Mix (working MMX) with DNA specimens, amplification and detection should be performed in an area separated from DNA Isolation. The amplification and detection work area should be thoroughly cleaned before working MMX preparation. For proper cleaning, all surfaces including racks and pipettors should be thoroughly wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution.
- K. **DNA PBB** and **WB I** contain guanidine hydrochloride. If liquid containing this buffer is spilled, clean with suitable laboratory detergent and water. If a spill occurs with potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 0.5% sodium hypochlorite*. If spills occur on the **cobas z 480** analyzer, follow the instructions in the **cobas z 480** analyzer Instrument Manual.

***NOTE: Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.**

- L. Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*⁹ and in the CLSI Document M29-A3.¹⁰
- M. **DNA PBB** contains Triton X-100, an irritant to mucous membranes. Avoid contact with eyes, skin, and mucous membranes.
- N. **DNA TLB, DNA EB, MGAC, EGFR MMX-1, EGFR MMX-2, EGFR MMX-3, EGFR MC, and DNA SD** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- O. Xylene is a hazardous chemical and should be used in a chemical hood. Discard into chemical waste in accordance with local, state, and federal regulations.
- P. Wear eye protection, laboratory coats, and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Q. All disposable items are for one time use. Do not reuse.
- R. Do not use disposable items beyond their expiration date.
- S. Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas z 480** analyzer. Clean the **cobas z 480** analyzer according to procedures described in the **cobas z 480** analyzer Instrument Manual.
- T. For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas z 480** analyzer, consult the **cobas z 480** analyzer Instrument Manual.
- U. The use of sterile disposable pipettes and DNase-free pipettor tips is recommended.

STORAGE AND HANDLING REQUIREMENTS

- A. With the exception of the **PK** reagent, do not freeze reagents.
- B. Store **DNA TLB, DNA PBB, WB I, WB II, DNA EB, PK, FT, and CT** at 15°C to 30°C. Once opened, **DNA TLB, DNA PBB, WB I, WB II, DNA EB, and PK** are stable for up to 8 uses over 90 days or until the expiration date, whichever comes first.
- C. After addition of sterile, nuclease free water to **PK**, store unused reconstituted **PK** in 450 µL aliquots at -20°C. Once reconstituted, **PK** must be used within 90 days or until the expiration date, whichever comes first.
- D. After addition of absolute ethanol, store **WB I** and **WB II** at 15°C to 30°C. These working solutions are stable for 90 days or until the expiration date, whichever comes first.
- E. Store **MGAC, EGFR MMX-1, EGFR MMX-2, EGFR MMX-3, EGFR MC, and DNA SD** at 2°C to 8°C. Once opened, these reagents are stable for 4 uses over 90 days or until the expiration date, whichever comes first.
- F. **EGFR MMX-1, EGFR MMX-2, EGFR MMX-3**, and working MMX (prepared by the addition of **MGAC** to **EGFR MMX-1 or EGFR MMX-2 or EGFR MMX-3**) should be protected from prolonged exposure to light.
- G. Working MMX must be stored at 2°C to 8°C in the dark. The prepared specimens and controls must be added within 1 hour of preparation of the working MMX.

- H. Processed specimens (extracted DNA) are stable for up to 24 hours at 15°C to 30°C or up to 14 days at 2°C to 8°C or up to 60 days at -15°C to -25°C or after undergoing 3 freeze thaws when stored at -15°C to -25°C. Extracted DNA should be amplified within the recommended storage periods or before the expiration date of the **cobas**[®] DNA Sample Preparation Kit used to extract the DNA, whichever comes first.
- I. Amplification must be started within 1 hour from the time that the processed specimens and controls are added to the working MMX (prepared by the addition of **MGAC** to **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3**).

MATERIALS PROVIDED

A. cobas[®] DNA Sample Preparation Kit
(P/N: 05985536190)

DNA SP

24 Tests

DNA TLB
(DNA Tissue Lysis Buffer)

PK
(Proteinase K)

DNA PBB
(DNA Paraffin Binding Buffer)

WB I
(DNA Wash Buffer I)

WB II
(DNA Wash Buffer II)

DNA EB
(DNA Elution Buffer)

FT
(Filter tubes with caps)

CT
(Collection Tubes)

B. cobas[®] EGFR Mutation Test
(P/N: 06471463190)

EGFR

24 Tests

MGAC
(Magnesium acetate) (Cap with Yellow Button)

EGFR MMX-1
(EGFR Master Mix 1) (Cap with White Button)

EGFR MMX-2
(EGFR Master Mix 2) (Cap with Gold Button)

EGFR MMX-3
(EGFR Master Mix 3) (Cap with Teal Button)

EGFR MC
(EGFR Mutant Control) (Cap with Red Button)

DNA SD
(DNA Specimen Diluent)

MATERIALS REQUIRED BUT NOT PROVIDED

- Xylene (ACS, ≥ 98.5% xylenes)
- Absolute ethanol (for Molecular Biology)
- Isopropanol (ACS, ≥ 99.5%)
- Sterile, nuclease-free water (For Molecular Biology)
- Sterile disposable, serological pipettes: 5 and 25 mL
- **cobas**[®] 4800 System Microwell Plate (AD-Plate) and Sealing Foil (Roche P/N 05232724001)
- **cobas**[®] 4800 Sealing Foil Applicator (Roche P/N 04900383001)
- Adjustable Pipettors* (capacity 10 µL, 20 µL, 200 µL, and 1000 µL) with aerosol barrier or positive displacement DNase-free tips
- Pipette aid (Drummond P/N: 4-000-100 or equivalent)
- Bench top microcentrifuge capable of 20,000 x g**

- Two (2) dry heat blocks capable of heating microcentrifuge tubes to 56°C and 90°C**
- 1.5 mL Safe-Lock microcentrifuge tubes, sterile, RNase/DNase free, PCR grade (Eppendorf, Cat# 022363212)
- Spectrophotometer for measuring DNA concentration **
- Vortex mixer**
- Microcentrifuge tube racks
- Disposable gloves, powder-free
- Calibrated thermometers for dry heat block**
- Waterbath** capable of maintaining 37°C
- Single edged blade or similar

* Pipettors should be maintained according to the manufacturer's instructions and accurate within 3% of stated volume. Aerosol barrier or positive displacement DNase-free tips must be used where specified to prevent specimen degradation and cross-contamination.

** All equipment should be properly maintained according to manufacturer's instructions.

Instrumentation and Software

- **cobas z 480** analyzer
- **cobas**® 4800 SR2 System Control Unit with OSXP image
- **cobas**® 4800 SR2 System Software version 2.0 or higher configured with the EGFR Analysis Package
- Barcode Reader ext USB
- Printer

SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

NOTE: Handle all specimens as if they are capable of transmitting infectious agents.

A. Specimen Collection

NSCLC FFPET specimens have been validated for use with the **cobas**® EGFR Mutation Test.

B. Specimen Transport

NSCLC FFPET specimens can be transported at 15°C to 30°C. Transportation of FFPET specimens must comply with country, federal, state, and local regulations for the transport of etiologic agents.¹²

C. Specimen Storage

Stability of FFPET specimens stored at 15-30°C for up to 12 months after the date of collection has been confirmed. 5 micron sections mounted on slides may be stored at 15-30°C for up to 60 days.

INSTRUCTIONS FOR USE

NOTE: Only NSCLC FFPET sections of 5 µm thickness containing at least 10% tumor content by area are to be used in the cobas® EGFR Mutation Test. Any specimen containing less than 10% tumor content by area should be macro-dissected after deparaffinization.

NOTE: Refer to the cobas z 480 analyzer Instrument Manual for detailed operating instructions for the cobas z 480 analyzer.

NOTE: Dry heat blocks capable of heating microcentrifuge tubes should be turned on and set at 56°C and 90°C.

Run Size

A single run can include from 1 to 30 specimens (plus controls) per 96 well Microwell plate. When running more than 24 specimens, multiple **cobas**® EGFR Mutation Test kits will be required.

The **cobas**® EGFR Mutation Test contains sufficient reagents for 8 runs of 3 specimens (plus controls) for a maximum of 24 specimens per kit.

Workflow

The **cobas**® EGFR Mutation Test consists of manual specimen preparation using the **cobas**® DNA Sample Preparation Kit followed by amplification/detection on the **cobas z 480** analyzer using the **cobas**® EGFR Mutation Test kit.

Reagent Preparation

- A. Reconstitute Proteinase K (**PK**) by adding 4.5 mL of sterile, nuclease-free (PCR grade) water to the vial using a sterile, disposable 5-mL serological pipette. Mix by inverting the vial 5 to 10 times. Aliquot 450 μ L of reconstituted **PK** into 1.5 mL Safe-Lock microcentrifuge tubes and store at -20°C . If the Proteinase K has already been reconstituted and frozen, thaw sufficient number of aliquots to process the number of specimens to be run prior to deparaffinization (70 μ L of reconstituted **PK** is required for each specimen).
- B. All solutions stored at $15\text{--}30^{\circ}\text{C}$ should be clear. If precipitate is present in any reagent, warm the solution in a 37°C water bath until the precipitate dissolves. Do not use until all precipitate has been dissolved.
- C. Prepare working DNA Wash Buffer I (**WB I**) by adding 15 mL of absolute ethanol to the bottle of **WB I**. Mix by inverting the bottle 5 to 10 times. Make a note on the bottle that ethanol has been added and the date. Store working **WB I** at 15°C to 30°C .
- D. Prepare working DNA Wash Buffer II (**WB II**) by adding 50 mL of absolute ethanol to the bottle of **WB II**. Mix by inverting the bottle 5 to 10 times. Make a note on the bottle that ethanol has been added and the date. Store working **WB II** at 15°C to 30°C .

Deparaffinization of FFPET Sections Mounted on Slides

NOTE: *Xylene is a hazardous chemical. All steps for deparaffinization should be performed under a chemical hood. See Warnings and Precautions.*

NOTE: *If the specimen contains less than 10% tumor content by area, the section must be macro-dissected*

- A. Add a slide with a mounted 5 μm FFPET section to a container with sufficient xylene to cover the tissue; soak for 5 minutes.
- B. Transfer the slide to a container with sufficient absolute ethanol to cover the tissue; soak for 5 minutes.
- C. Remove the slide from the ethanol and allow the section to air dry completely (5 to 10 minutes).
- D. Perform macro-dissection if the specimen contains less than 10% tumor content by area.
- E. Label one 1.5 mL Safe-Lock microcentrifuge tube for each specimen with the specimen identification information.
- F. Add 180 μL **DNA TLB** to the 1.5-mL Safe-Lock microcentrifuge tube.
- G. Add 70 μL of reconstituted **PK** to the Safe-Lock tube containing **DNA TLB**.
- H. Scrape the tissue off the slide and into the Safe-Lock tube. Immerse the tissue in the **DNA TLB/PK** mixture.
- I. Continue with Step A of the DNA Isolation procedure.

Deparaffinization of FFPET Sections not Mounted on Slides

NOTE: *Xylene is a hazardous chemical. All steps for deparaffinization should be performed under a chemical hood. See Warnings and Precautions.*

NOTE: *If the specimen contains less than 10% tumor content by area, the section must be mounted on a slide for macro-dissection and the procedure detailed in 'Deparaffinization of FFPET Sections Mounted on Slides' must be followed.*

- A. Place one 5-micron FFPET section into a 1.5 mL Safe-Lock microcentrifuge tube labeled with the specimen identification information for each specimen.
- B. Add 500 μL Xylene to the Safe-Lock tube containing the FFPET section.
- C. Mix well by vortexing for 10 seconds.
- D. Let the tube stand for 5 minutes at 15°C to 30°C .
- E. Add 500 μL absolute ethanol and mix by vortexing for 10 seconds.
- F. Let the tube stand for 5 minutes at 15°C to 30°C .
- G. Centrifuge at $16,000 \times g$ to $20,000 \times g$ for 2 minutes. Remove the supernatant without disturbing the pellet. Discard the supernatant into chemical waste.
- H. Add 1 mL absolute ethanol and vortex for 10 seconds.
- I. Centrifuge at $16,000 \times g$ to $20,000 \times g$ for 2 minutes. Remove the supernatant without disturbing the pellet. Discard the supernatant into chemical waste.

NOTE: *If the pellet is floating in the remaining supernatant, spin again for 1 minute at $16,000 \times g$ to $20,000 \times g$. Remove any remaining supernatant.*

- J. Dry the tissue pellet for 10 minutes at 56°C in a heating block with the tube open.

NOTE: *Make sure the ethanol is completely evaporated and the pellet is dry before proceeding to the next step.*

NOTE: *If needed, dry pellets can be stored up to 24 hours at 2°C to 8°C .*

- K. Resuspend the tissue pellet in 180 µL DNA Tissue Lysis Buffer (**DNA TLB**).
- L. Add 70 µL of reconstituted **PK**.
- M. Continue with Step A of the DNA Isolation procedure.

SPECIMEN PREPARATION

DNA Isolation Procedure

NOTE: Process a negative control concurrently with the specimen(s). Prepare the negative control by combining 180 µL DNA Tissue Lysis Buffer (DNA TLB) and 70 µL PK solution in a 1.5 mL Safe-Lock microcentrifuge tube labeled as NEG CT. The negative control should be processed following the same procedure as the specimens.

- A. Vortex the tubes containing the specimen/**DNA TLB/PK** mixture and the negative control mixture (**NEG CT**) for 30 seconds.

NOTE: The tissue must be fully immersed in the DNA TLB/PK mixture.

- B. Place tubes in the 56°C dry heat block and incubate for 60 minutes.
- C. Vortex the tubes for 10 seconds.

NOTE: The tissue must be fully immersed in the DNA TLB/PK mixture.

- D. Place tubes in the 90°C dry heat block and incubate for 60 minutes.

NOTE: During the incubation, prepare the required number of filter tubes (FTs) with hinged caps by placing the FT onto a collection tube (CT) and labeling each FT cap with the proper specimen or control identification.

NOTE: Each specimen will need 1 FT, 3 CTs and 1 elution tube (1.5 mL microcentrifuge tube).

NOTE: During the incubation, label the required number of elution tubes (1.5 mL microcentrifuge tube) with the proper specimen or control identification information.

- E. Allow the tubes to cool to 15°C to 30°C. After cooling, pulse-centrifuge the tubes to collect liquid from the caps.
- F. Add 200 µL **DNA PBB** to each tube; mix by pipetting up and down 3 times.
- G. Incubate the tubes at 15°C to 30°C for 10 minutes.
- H. Add 100 µL isopropanol to each tube; mix lysate by pipetting up and down 3 times.
- I. Transfer each lysate into the appropriately labeled **FT/CT** unit.
- J. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
- K. Place each **FT** onto a new **CT**. Discard the flow-through from the old **CT** into chemical waste, and properly dispose of the used **CT**.
- L. Add 500 µL working **WB I** to each **FT**.

NOTE: Preparation of working WB I is described in the Reagent Preparation section.

- M. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
- N. Discard the flow-through in each **CT** into chemical waste. Place the **FT** back into the same **CT**.
- O. Add 500 µL working **WB II** to each **FT**.

NOTE: Preparation of working WB II is described in the Reagent Preparation section.

- P. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
- Q. Place each **FT** onto a new **CT**. Discard the flow-through from the old **CT** into chemical waste, and properly dispose of the used **CT**.
- R. Centrifuge the **FT/CT** units at 16,000 to 20,000 x g for 1 minute to dry the filter membranes.
- S. Place each **FT** into an elution tube (1.5 mL microcentrifuge tube) pre-labeled with specimen or control identification. Discard the flow-through from the used **CT** into chemical waste, and properly dispose of the used **CT**.
- T. Add 100 µL **DNA EB** to the center of each **FT** membrane without touching the **FT** membrane.
- U. Incubate the **FT** with elution tube at 15°C to 30°C for 5 minutes.
- V. Centrifuge the **FT** with elution tube at 8,000 x g for 1 minute to collect eluate into the elution tube. Properly dispose of the used **FT**.
- W. Close the cap on the elution tube. The elution tube contains the DNA Stock. Proceed to Step A in the **DNA Quantitation** section.

NOTE: Measurement of DNA concentration should be performed immediately after the DNA Isolation procedure and prior to storage.

DNA Quantitation:

- A. Mix each DNA Stock by vortexing for 5 seconds.
- B. Quantify DNA using a spectrophotometer according to the manufacturer's protocol. Use **DNA EB** as the blank for the instrument. An average of two consistent readings is necessary. The two measurements should be within $\pm 10\%$ of each other when the DNA concentration readings are ≥ 20.0 ng/ μ L. For DNA concentration readings < 20.0 ng/ μ L, the two measurements should be within ± 2 ng/ μ L. If the two measurements are not within $\pm 10\%$ of each other when the DNA concentration readings are ≥ 20.0 ng/ μ L or within ± 2 ng/ μ L when the DNA concentration readings are < 20.0 ng/ μ L, an additional 2 readings must be taken until the requirements are met. The average of these two new measurements should then be calculated.

NOTE: *The DNA Stock from the processed negative control (NEG CT) does not need to be measured.*

- C. The DNA Stock concentration from the specimens must be ≥ 2 ng/ μ L to perform the **cobas**[®] EGFR Mutation Test. Three amplification/detections are run per specimen, using 25 μ L of a 2 ng/ μ L dilution of DNA Stock (total of 50 ng DNA) for each amplification/detection.

NOTE: *Each DNA Stock must have a minimum concentration of 2 ng/ μ L to perform the cobas[®] EGFR Mutation Test. If the concentration of a DNA Stock is < 2 ng/ μ L, repeat the deparaffinization, DNA Isolation, and DNA Quantitation procedures for that specimen using two 5 μ m FFPET sections. For mounted specimens, after deparaffinization, combine the tissue from both sections into one tube, immerse the tissue in DNA TLB + PK, and perform DNA Isolation and Quantitation as described above. For unmounted specimens, combine two sections into one tube and immerse the tissue in DNA TLB + PK, and perform DNA Isolation and Quantitation as described above. If the DNA Stock is still < 2 ng/ μ L, request another FFPET specimen section from the referring clinical site.*

NOTE: *Processed specimens (extracted DNA) are stable for up to 24 hours at 15°C to 30°C or up to 14 days at 2°C to 8°C or up to 60 days at -15°C to -25°C or after undergoing 3 freeze thaws when stored at -15°C to -25°C. Extracted DNA should be amplified within the recommended storage periods or before the expiration date of the cobas[®] DNA Sample Preparation Kit used to extract the DNA, whichever comes first.*

AMPLIFICATION AND DETECTION

NOTE: *To avoid contamination of working MMX with DNA specimens, amplification and detection should be performed in an area separated from DNA Isolation. The amplification and detection work area should be thoroughly cleaned before working MMX preparation. For proper cleaning, all surfaces including racks and pipettors should be thoroughly wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.*

Instrument Set-Up:

Refer to the **cobas z** 480 analyzer Instrument Manual for detailed instruction for the **cobas z** 480 set up.

Test Order Set-up:

Refer to the **cobas**[®] 4800 system Operator's Manual Software Version 2.0 for **cobas**[®] EGFR Mutation Test (**cobas**[®] 4800 EGFR Operator's Manual) for detailed instructions on the EGFR workflow steps.

Dilution Calculation of Specimen DNA Stock:

Dilution Calculation for DNA Stock Concentrations from 2 ng/ μ L to 36 ng/ μ L

NOTE: *DNA stocks from specimens should be diluted immediately prior to amplification and detection.*

NOTE: *Three (3) amplification/detections are run for each specimen requiring a total volume of 75 μ L (25 μ L for each of three reactions) of a 2 ng/ μ L dilution of DNA Stock (total of 150 ng DNA).*

- A. For each specimen, calculate the volume (μ L) of DNA stock needed:

$$\mu\text{L of DNA stock} = (90 \mu\text{L} \times 2 \text{ ng}/\mu\text{L}) \div \text{DNA Stock concentration [ng}/\mu\text{L}]$$

- B. For each specimen, calculate the volume (μ L) of DNA Specimen Diluent (**DNA SD**) needed:

$$\mu\text{L of DNA SD} = 90 \mu\text{L} - \mu\text{L of DNA Stock}$$

Example:

$$\text{DNA stock concentration} = 6.5 \text{ ng}/\mu\text{L}$$

A. $\mu\text{L of DNA Stock} = (90 \mu\text{L} \times 2 \text{ ng}/\mu\text{L}) \div 6.5 \text{ ng}/\mu\text{L} = 27.7 \mu\text{L}$

B. $\mu\text{L of DNA SD} = (90 \mu\text{L} - 27.7 \mu\text{L}) = 62.3 \mu\text{L}$

Dilution Calculation for DNA Stock Concentrations > 36 ng/ μ L

NOTE: *DNA Stocks from specimens should be diluted immediately prior to amplification and detection.*

NOTE: *Three (3) amplification/detections are run for each specimen requiring a total volume of 75 μ L (25 μ L for each of three reactions) of a 2 ng/ μ L dilution of DNA stock (total of 150 ng DNA).*

- A. At DNA Stock concentrations > 36 ng/μL, use the following formula to calculate the amount of DNA Specimen Diluent (**DNA SD**) required to prepare at least 90 μL of diluted DNA stock. This is to ensure that each specimen uses a minimum of 5 μL of DNA stock.
- B. For each specimen, calculate the volume (μL) of **DNA SD** needed to dilute 5 μL of DNA Stock to 2 ng/μL:
 Vol. of **DNA SD** required in μL = [(5 μL of DNA stock x DNA stock concentration in ng/μL) / 2 ng/μL] – 5 μL

Example:

DNA stock concentration = 100 ng/μL

- A. Vol. of **DNA SD** required in μL = [(5 μL x 100 ng/μL) / 2 ng/μL] – 5 μL = 245 μL
- B. Use the calculated volume of **DNA SD** to dilute 5 μL of DNA stock.

Specimen Dilution

- A. Prepare the appropriate number of 1.5 mL Safe-Lock microcentrifuge tubes for DNA Dilutions by labeling them with the proper specimen identification.
- B. Using a pipettor with an aerosol-resistant tip, pipette the calculated volumes of **DNA SD** into the respectively labeled tubes. Pipette 45 μL of **DNA SD** into a Safe-Lock tube labeled as **NEG CT**.
- C. Vortex each DNA stock and the negative control for 5 to 10 seconds.
- D. Using a pipettor with an aerosol-resistant pipette tip (new tip for each pipetting), gently pipette the calculated volume of each DNA stock into the respective tube containing **DNA SD**. Pipette 45 μL of negative control (extracted eluate) into the **NEG CT** tube.
- E. Cap the tubes and vortex each for 5 to 10 seconds.
- F. Change gloves.

Preparation of Working Master Mixes (MMX-1, MMX-2 and MMX-3)

NOTE: EGFR MMX-1, EGFR MMX-2, EGFR MMX-3, and working MMX are light-sensitive and must be protected from prolonged exposure to light.

NOTE: Due to the viscosity of the EGFR MIXES and working MMX, pipette slowly to ensure all mix is completely dispensed from the tip.

NOTE: The EGFR MMX-1, EGFR MMX-2, and EGFR MMX-3 may appear light blue/purplish. This does not affect the performance of the reagent.

Prepare three bulk working MMX, one containing **EGFR MMX-1**, one containing **EGFR MMX-2**, and the other containing **EGFR MMX-3** in separate 1.5 mL Safe-Lock microcentrifuge tubes.

- A. Calculate the volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3** required for each working MMX using the following formula:
 Volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3** required = (Number of Specimens + 2 Controls + 1) x 20 μL
- B. Calculate the volume of **MGAC** required for each working MMX using the following formula:
 Volume of **MGAC** required = (Number of Specimens + 2 Controls + 1) x 5 μL

Use Table 1 to determine the volume of each reagent needed for the preparation of working MMX based on the number of specimens included in the run.

**Table 1:
 Volumes of Reagents Needed for Working MMX-1, Working MMX-2 and Working MMX-3**

		# of Specimens*									
		1	2	3	4	5	6	7	8	9	10
MMX	20 μL	80	100	120	140	160	180	200	220	240	260
MGAC	5 μL	20	25	30	35	40	45	50	55	60	65
Total Vol. for Each Working MMX (μL)		100	125	150	175	200	225	250	275	300	325

* Volumes for # of Specimens is based on the sum of the # Specimens + 2 Controls + 1

- C. Remove the appropriate number of **EGFR MMX-1**, **EGFR MMX-2**, **EGFR MMX-3**, and **MGAC** vials from 2°C to 8°C storage. Vortex each reagent for 5 seconds and collect liquid at the bottom of the tube before use. Label a sterile microcentrifuge tube for working MMX-1, working MMX-2, and working MMX-3.
- D. Add the calculated volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3** to their respective working MMX tube.
- E. Add the calculated volume of **MGAC** to the working MMX tubes.
- F. Vortex the tubes for 3 to 5 seconds to ensure adequate mixing.

NOTE: Specimens and controls should be added to the microwell plate (AD-plate) within 1 hour after the preparation of the working MMXs.

NOTE: Use only cobas® 4800 System Microwell Plate (AD-Plate) and Sealing Foil (Roche P/N 05232724001).

Preparation of Plate

**Figure 1:
Sample Plate Layout**

Figure 1: Plate layout for the cobas® EGFR Mutation Test

Row / Column	1	2	3	4	5	6	7	8	9	10	11	12
A	MUT MMX-1	MUT MMX-2	MUT MMX-3	S7 MMX-1	S7 MMX-2	S7 MMX-3	S15 MMX-1	S15 MMX-2	S15 MMX-3	S23 MMX-1	S23 MMX-2	S23 MMX-3
B	NEG MMX-1	NEG MMX-2	NEG MMX-3	S8 MMX-1	S8 MMX-2	S8 MMX-3	S16 MMX-1	S16 MMX-2	S16 MMX-3	S24 MMX-1	S24 MMX-2	S24 MMX-3
C	S1 MMX-1	S1 MMX-2	S1 MMX-3	S9 MMX-1	S9 MMX-2	S9 MMX-3	S17 MMX-1	S17 MMX-2	S17 MMX-3	S25 MMX-1	S25 MMX-2	S25 MMX-3
D	S2 MMX-1	S2 MMX-2	S2 MMX-3	S10 MMX-1	S10 MMX-2	S10 MMX-3	S18 MMX-1	S18 MMX-2	S18 MMX-3	S26 MMX-1	S26 MMX-2	S26 MMX-3
E	S3 MMX-1	S3 MMX-2	S3 MMX-3	S11 MMX-1	S11 MMX-2	S11 MMX-3	S19 MMX-1	S19 MMX-2	S19 MMX-3	S27 MMX-1	S27 MMX-2	S27 MMX-3
F	S4 MMX-1	S4 MMX-2	S4 MMX-3	S12 MMX-1	S12 MMX-2	S12 MMX-3	S20 MMX-1	S20 MMX-2	S20 MMX-3	S28 MMX-1	S28 MMX-2	S28 MMX-3
G	S5 MMX-1	S5 MMX-2	S5 MMX-3	S13 MMX-1	S13 MMX-2	S13 MMX-3	S21 MMX-1	S21 MMX-2	S21 MMX-3	S29 MMX-1	S29 MMX-2	S29 MMX-3
H	S6 MMX-1	S6 MMX-2	S6 MMX-3	S14 MMX-1	S14 MMX-2	S14 MMX-3	S22 MMX-1	S22 MMX-2	S22 MMX-3	S30 MMX-1	S30 MMX-2	S30 MMX-3

Where: NEG = Negative Control, MUT = Mutant Control, S# = sample ID, and MMX-# corresponds to Master Mix Reagent 1, 2, or 3.

NOTE: Any given sample must be spread across three consecutive columns in one row in order to generate a result.

- A. Pipette 25 µL of working MMX into each reaction well of the microwell plate (AD-plate) that is needed for the run. Do not allow the pipettor tip to touch the plate outside the well.
 - Add working MMX-1 (containing **EGFR MMX-1**) to the microwell plate (AD-plate) wells in columns 1, 4, 7, and 10, as needed.
 - Add working MMX-2 (containing **EGFR MMX-2**) to the microwell plate (AD-plate) wells in columns 2, 5, 8, and 11, as needed.
 - Add working MMX-3 (containing **EGFR MMX-3**) to the microwell plate (AD-plate) wells in columns 3, 6, 9, and 12, as needed.
- B. Pipette 25 µL of **EGFR MC** into wells **A1**, **A2**, and **A3** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.
- C. Using a new pipettor tip, pipette 25 µL of **NEG CT** into wells **B1**, **B2**, and **B3** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.

NOTE: Each run must contain positive control (EGFR MC) in wells A1, A2 and A3, and negative control (NEG CT) in wells B1, B2, and B3 or the run will be invalidated by the cobas z 480 analyzer.

NOTE: Change gloves as needed to protect against specimen-to-specimen contamination and external PCR reaction tube contamination.

- D. Using new pipettor tips for each diluted specimen DNA, add 25µL of the first specimen DNA to wells **C1**, **C2**, and **C3** of the microwell plate (AD-plate), using a new tip for the addition of the specimen DNA to each well; mix each well using a pipette to aspirate and dispense within the well a minimum of two times. Repeat this procedure for the diluted DNA from the second specimens (wells **D1**, **D2**, and **D3**). Follow the template in Figure 1 until all specimens' DNA Dilutions are loaded onto the microwell plate (AD-plate). Ensure that all liquid is collected at the bottom of the wells.
- E. Cover the microwell plate (AD-plate) with sealing foil (supplied with the plates). Use the sealing foil applicator to seal the foil firmly to the microwell plate (AD-plate).
- F. Confirm that all liquid is collected at the bottom of each well before starting PCR.

NOTE: Amplification and Detection should be started within 1 hour after the addition of the first specimen DNA dilution to the working MMX.

Starting PCR

Refer to the **cobas**[®] EGFR Operator's Manual for detailed instructions on the EGFR workflow steps.

INTERPRETATION OF RESULTS

NOTE: All run and specimen validation is performed by the **cobas**[®] 4800 software.

NOTE: A valid test run may include both valid and invalid sample results.

For a valid run, specimen results are interpreted as shown in Table 2.

Table 2:
Result Interpretation of **cobas[®] EGFR Mutation Test**

Test Result	Mutation Result	Interpretation
Mutation Detected	Exon 19 Deletion Exon 21 L858R (More than one mutation may be present)	Mutation detected in specified targeted EGFR region.
Mutation Not Detected*	N/A	Mutation not detected in targeted EGFR regions
Invalid	N/A	Specimen result is invalid. Repeat the testing of specimens with invalid results following the instructions outlined in the “ Retesting of Specimens with Invalid Results ” section below.
Failed	N/A	Failed run due to hardware or software failure. Contact your local Roche office for technical assistance

* A Mutation Not Detected result does not preclude the presence of a mutation in the targeted EGFR regions because results depend on percent mutant sequences, adequate specimen integrity, absence of inhibitors, and sufficient DNA to be detected.

Retesting of Specimens with Invalid Results

A. Repeat dilution of the invalid specimen DNA stock starting from “**Dilution Calculation of Specimen DNA Stock**” and “**Specimen Dilution**” procedures in the “**AMPLIFICATION and DETECTION**” section.

B. After performing the DNA stock dilution to 2 ng/μL as described in “**Specimen Dilution**”, continue with “**Preparation of Working Master Mix (working MMX)**” and the remainder of the amplification and detection procedure.

NOTE: If the specimen remains invalid after retesting or there was not enough DNA stock to prepare another dilution in **Retesting of Specimens with Invalid Results**, step A, repeat the entire test procedure for that specimen, starting with **Deparaffinization and DNA Isolation using a new 5-micron FFPE tumor section**.

QUALITY CONTROL

One set of **cobas**[®] EGFR Test Mutant Control (**EGFR MC**) (wells **A1**, **A2** and **A3**) and negative control (**NEG CT**) (wells **B1**, **B2** and **B3**) for working MMX-1, working MMX-2, and working MMX-3 are included in each run of up to 30 specimens. A run is valid if the EGFR Mutant Control (**EGFR MC**) and the negative control (**NEG CT**) are valid. If an EGFR Mutant Control (**EGFR MC**) or negative control (**NEG CT**) is invalid, the entire run is invalid and must be repeated. Prepare a fresh dilution of the previously isolated specimen DNA Stock to set up a new microwell plate (AD-plate) with controls for amplification and detection.

Mutant Control

The EGFR Mutant Control (**EGFR MC**) result must be 'Valid'. If the **EGFR MC** results are consistently invalid, contact your local Roche office for technical assistance.

Negative Control

The negative control (**NEG CT**) result must be 'Valid'. If the **NEG CT** results are consistently invalid, contact your local Roche office for technical assistance.

PROCEDURAL PRECAUTIONS

As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

PROCEDURAL LIMITATIONS

1. Test only the indicated specimen types. The **cobas**[®] EGFR Mutation Test has only been validated for use with NSCLC FFPE tumor specimens.
2. The **cobas**[®] EGFR Mutation Test has only been validated using the **cobas**[®] DNA Sample Preparation Kit (Roche P/N: 05985536190).
3. Detection of a mutation is dependent on the number of copies present in the specimen and may be affected by specimen integrity, amount of isolated DNA, and the presence of interfering substances.

4. Reliable results are dependent on adequate specimen fixation, transport, storage and processing. Follow the procedures in this Package Insert and in the **cobas**[®] EGFR Operator's Manual.
5. The effects of other potential variables such as specimen fixation variables have not been evaluated.
6. The addition of AmpErase enzyme into the **cobas**[®] EGFR Mutation Test Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.
7. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**[®] 4800 system.
8. Only the **cobas z** 480 analyzer has been validated for use with this product. No other thermal cycler with real-time optical detection can be used with this product.
9. The presence of PCR inhibitors may cause false negative or invalid results.
10. Though rare, mutations within the genomic DNA regions of the EGFR gene covered by the primers or probes used in the **cobas**[®] EGFR Mutation Test may result in failure to detect presence of a mutation in exons 19 and 21 (results of "Mutation Not Detected").
11. The **cobas**[®] EGFR Mutation Test shows cross-reactivity (results of "Mutation Detected") to the exon 19 L747S mutation, a rare acquired mutation that may confer resistance to TKI treatment.¹²
12. The **cobas**[®] EGFR Mutation Test shows cross-reactivity (results of "Mutation Detected") to additional rare exon 19 deletion mutations, and exon 21 L858R substitution mutation. Refer to Table 6 and 7 in the **Non-clinical Performance Evaluation** section below for more details.
13. The **cobas**[®] EGFR Mutation Test is validated for use with 50 ng of DNA per reaction well. DNA input amounts lower than 50 ng per reaction well are not recommended.
14. The **cobas**[®] EGFR Mutation Test is a qualitative test. The test is not for quantitative measurements of percent mutation.
15. NSCLC FFPET specimens containing degraded DNA may affect the ability of the test to detect the EGFR mutations.
16. Samples with results reported as "Mutation Not Detected" may harbor EGFR mutations not detected by the assay.
17. The **cobas**[®] EGFR Mutation Test detects EGFR mutations in NSCLC patients whose tumors have the exon 19 deletions or exon 21 L858R substitution mutations, but not any other EGFR mutations.

NON-CLINICAL PERFORMANCE EVALUATION

For the non-clinical studies described below, percentage of tumor was assessed by pathology review. Bi-directional Sanger sequencing and massively parallel sequencing (MPS) were used to select the specimens for testing. Percentage of mutation of NSCLC FFPET specimen was determined using a MPS method.

Analytical Sensitivity – Limit of Blank

To assess performance of the **cobas**[®] EGFR Mutation Test in the absence of template and to ensure that a blank sample does not generate an analytical signal that might indicate a low concentration of mutation, samples with no template and NSCLC FFPET EGFR wild-type specimens were evaluated. No detectable Ct results were identified in the mutant channel in the presence of EGFR wild-type DNA or in either channel following a no template sample.

Analytical Sensitivity Using FFPET Specimen Blends

Eleven FFPET specimen DNA extracts for the exon 19 Deletion mutations and four FFPET specimen DNA extracts for the L858R mutation were blended with EGFR wild-type FFPET specimen extracts to achieve blends with samples targeting 10, 5.0, 2.5 and 1.25% mutation level as determined by a massively parallel sequencing method (MPS), that was validated for the use for detecting the EGFR exon 19 deletions and exon 21 L858R mutation. Serial dilutions of each specimen blend were prepared and eight (8) replicates of each panel member were run using each of 3 **cobas**[®] EGFR Mutation Test kit lots (n=24/panel member). The sensitivity of each sample was determined by the lowest amount of DNA that gave an EGFR "Mutation Detected" rate of at least 95% for the targeted mutation, shown in Table 3.

Table 3:
Sensitivity of the cobas[®] EGFR Mutation Test using FFPET Specimen Blends

EGFR Mutation	EGFR Nucleic Acid Sequence	Percent Mutation in the Panel Member to achieve ≥95% “Mutation Detected” Rate with 50 ng DNA input per reaction well (N=24 replicates)
Exon 19 Deletion	2235_2249del15	1.4
	2236_2250del15	2.5
	2238_2252del15	2.4 [#]
	2239_2248>C	2.2
	2240_2254del15	7.2
	2240_2257del18	13.4 ^{**}
	2237_2253>TTGCT*	6.32
	2237_2255>T*	4.08
	2239_2256del18*	4.74
	2238_2252del15*	5.45 [#]
	2239_2257>GT*	6.02
L858R ⁺	2573 T>G	4.0
	2573 T>G	4.2
	2573 T>G	4.3
	2573 T>G	5.3

* Only target levels of approximately 5% mutation was tested for these non-predominant exon 19 deletion mutations present in the EURTAC cohort. Specimen DNA blends were tested across 3 study sites.

** Analytical sensitivity of the cobas[®] EGFR Mutation Test for detecting this mutation is greater than 10% mutation level using the standard input of 50 ng per reaction well.

#Two independent specimens for the exon 19 deletion (2238_2252del15) were tested.

+ Four independent L858R specimens were tested

This study demonstrates that the cobas[®] EGFR Mutation Test can detect mutations in EGFR exons 19 and 21 with at least 5% mutation level using the standard input of 50 ng per reaction well.

Minimal Tumor Content

A total of 66 independent EGFR mutant specimens (i.e., 35 of exon 19 deletion mutants and 31 exon 21 L858R mutants) with tumor content ranging from 25% to 99% were tested to determine the minimum tumor content required for detecting the EGFR mutation in NSCLC specimens. None of the specimens evaluated had both an exon 19 deletion mutation and an exon 21 L858R mutation. Each specimen was tested without macrodissection (neat), and after macrodissection. The observed CtR values for the neat and macrodissected slides were analyzed using Deming regression and the Bland-Altman plot (differences vs. mean). The results support the use of samples whose tumor content is greater than 25% without macrodissection.

In the Phase 3 EURTAC trial of erlotinib vs. cisplatin-based chemotherapy, NSCLC FFPET specimens with less than 10% tumor content were macro-dissected prior to EGFR mutation analysis. A subset of the EURTAC screened samples was evaluated for EGFR mutation status by both the cobas[®] EGFR Mutation Test and the massively parallel sequencing (MPS) methods. Table 4 and Table 5 below included NSCLC specimens with valid paired results of EGFR Exon 19 or L858R mutations combined from both the cobas EGFR Mutation Test and the MPS sequencing. Using the MPS as the reference method, results showed that macro-dissection of NSCLC FFPET sections with less than 10% tumor content demonstrated comparable analytical accuracy to NSCLC FFPET section without macro-dissection.

Together, these studies support that macrodissection is required for NSCLC FFPET sections with less than 10% tumor content prior to testing with the cobas[®] EGFR Mutation Test.

Table 4: Performance of the cobas® EGFR Mutation Test for NSCLC FFPET Specimens with Tumor Contents ≤ 10% (Macro-dissected)

cobas® EGFR Mutation Test (Test Method)	MPS Sequencing (Reference Method)		
	Mutation Detected ^a	Mutation Not Detected ^b	Total
Mutation Detected	35	3	38
Mutation Not Detected	1	52	53
Total	36 (39.6%)	55 (60.4%)	91
PPA (95% CI)	35/36 = 97.2% (85.8%, 99.5%)		
NPA (95% CI)	52/55 = 94.5% (85.1%, 98.1%)		
OPA (95% CI)	87/91 = 95.6% (89.2%, 98.3%)		

Table 5: Performance of the cobas® EGFR Mutation Test for NSCLC FFPET Specimens with Tumor Contents > 10% (Not Macro-dissected)

cobas® EGFR Mutation Test (Test Method)	MPS Sequencing (Reference Method)		
	Mutation Detected ^a	Mutation Not Detected ^b	Total
Mutation Detected ^a	107	3	110
Mutation Not Detected ^b	8	199	207
Valid Total	115 (36.3%)	202 (63.7%)	317
PPA (95% CI)	107/115 = 93.0% (86.9%, 96.4%)		
NPA (95% CI)	199/202 = 98.5% (95.7%, 99.5%)		
OPA (95% CI)	306/317 = 96.5% (93.9%, 98.1%)		

^a Mutation Detected indicates the presence of EGFR Exon 19 or L858R Mutations Combined.

^b Mutation Not Detected indicates the absence of EGFR Exon 19 or L858R Mutations Combined.

Note: CI = (score) confidence interval.

Cross-Reactivity to Other Exon 19 and Exon 21 Mutations

EURTAC Clinical Trial Specimens

The cobas® EGFR Mutation Test gave “Mutation Detected” results for the following EGFR mutations observed in the EURTAC clinical trial specimens. Analytical performance of the cobas® EGFR Mutation Test in detecting these mutations has not been evaluated.

Table 6: Mutations Observed in the EURTAC Cohort Determined to Cross-React with the cobas® EGFR Mutation Test

Exon 19 – Mutation Call Exon 19 Deletion		
Mutation	AA Change	COSMIC ID ¹³
2234_2251>AAT	K745_T751>K	Not Found
2236_2244del9	E746_R748>E	Not Found
2236_2252>AT	E746_T751>I	26680
2236_2263>GAAGCAT	E746_A755>E	Not Found
2237_2251>AAC	E746_751T>E	Not Found
2239_2253>CAA	L747_T751>Q	51527
2237_2257>TCT	E746_P753>VS	18427
2239_2251>C	L747_T751>P	12383

Plasmid Samples

The cobas® EGFR Mutation Test has been shown to cross-react with the following mutations shown in Table 7. Plasmid constructs containing the non-predominant mutation for exon 19 deletions (n=19) and exon 21 L858R mutation (n=1) were blended with wild-type genomic DNA to create 5% mutant sample with 50 ng DNA input per PCR. Results demonstrated that the cobas® EGFR

Mutation Test cross-reacts to the following mutations at a $\geq 86\%$ hit rate. Analytical performance of the **cobas**[®] EGFR Mutation Test in detecting these mutations using FFPE NSCLC specimens has not been evaluated.

Table 7:
Mutations Determined to Cross-React with the cobas[®] EGFR Mutation Test

Exon 19 – Mutation Call Exon 19 Deletion		
Mutation	AA Change	COSMIC ID¹³
2237_2251del15	E746_T751>A	12678
2239_2253del15	L747_T751del	6254
2239_2247del9	L747_E749del	6218
2235_2252>AAT	E746_T751>I	13551
2236_2253del18	E746_T751del	12728
2237_2254del18	E746_S752>A	12367
2238_2255del18	E746_S752>D	6220
2238_2248>GC	L747_A750>P	12422
2238_2252>GCA	L747_T751>Q	12419
2239_2258>CA	L747_P753>Q	12387
2240_2251del12	L747_T751>S	6210
2233_2247del15	K745_E749del	26038
2253_2276del24	S752_I759del	13556
2235_2248>AATTC	E746_A750>IP	13550
2237_2252>T	E746_T751>V	12386
2235_2251>AATTC	E746_T751>IP	13552
2235_2255>AAT	E746_S752>I	12385
2239_2256>CAA	L747_S752>Q	12403
2240T>C	L747S	26704
Exon 21 –Mutation Call L858R		
Mutation	AA Change	COSMIC ID¹³
2573_2574TG>GT	L858R	12429

Specificity – Microorganisms and EGFR Homologs

Specificity of the **cobas**[®] EGFR Mutation Test was evaluated by testing lung-related microorganisms, and plasmids of EGFR homologs, i.e., plasmids containing the sequences from each of the HER2, HER3, and HER4 genetic regions analogous to the sequences in EGFR exons 19 and 21 amplified by the **cobas**[®] EGFR Mutation Test.

Lung-related Microorganisms

Streptococcus pneumoniae and *Haemophilus influenzae* at 1×10^6 colony forming units were found not to cross react or interfere with the **cobas**[®] EGFR Mutation Test when added to specimens containing wild-type and mutant EGFR sequences during the tissue lysis step.

Plasmids of EGFR Homologs

Structurally related epidermal receptor tyrosine kinase protein analog sequences (EGFR/HER1, HER2, HER3 and HER4) have been shown not to cross-react with the **cobas**[®] EGFR Mutation Test when the potential cross-reactive sequence was added at a genomic copy number equivalent to 50 ng/PCR input to the isolated DNA stock prior to the amplification/detection procedure. A control condition without plasmid DNA was included. Results indicated that the observed mutations for all 10 tested FFPE specimens matched the expected mutation as determined by sequencing, in the presence and absence of the added HER gene plasmid DNA. Additionally, the EGFR exon 19 mutation L747S was tested for cross reactivity. Results indicated that the **cobas**[®] EGFR Mutation Test cross-reacts with the EGFR exon 19 mutation L747S.

Interference

Triglycerides ($\leq 37\text{mM}$, CLSI recommended high concentration¹⁴) and hemoglobin ($\leq 2 \text{ mg/mL}$, CLSI recommended high concentration¹⁴) have been shown not to interfere with the **cobas**[®] EGFR Mutation Test when the potential interfering substance was added to the lysis step during the specimen preparation procedure.

Necrotic Tissue

NSCLC FFPET specimens with necrotic tissue content up to 60% for EGFR mutant and 85% in wild-type specimens have been shown not to interfere with the call results using the **cobas**[®] EGFR Mutation Test.

Repeatability

Repeatability of the **cobas**[®] EGFR Mutation Test was assessed using six FFPET specimens, including: 4 wild-type specimens; 2 mutant specimens with exon 19 deletion and L858R mutations respectively. These specimens were tested in duplicate by two operators, using two different reagent lots and two **cobas z** 480 analyzers over 4 days. A total of 32 replicates were evaluated per sample. The **cobas**[®] EGFR Mutation Test had a correct call rate of 99% (190/192).

Specimen Handling Reproducibility

The reproducibility of the DNA Sample Preparation Kit was examined using sections taken from three FFPET specimen blocks, one containing an exon 19 deletion mutation, one containing an L858R mutation, and one that is wild-type. Each specimen was tested in duplicate at each site on each day. The specimen sections for a given specimen were randomized and tested over a six day period across three sites using one operator at each site, one **cobas z** 480 analyzer at each site, three **cobas**[®] DNA Sample Preparation Kit lots, and one **cobas** EGFR Mutation Test kit lots. On each test day, each operator isolated and tested the DNA from two NSCLC FFPET curl sections for each specimen using the **cobas**[®] EGFR Mutation Test. All specimens reported valid and correct results through-out the six days of testing. For all specimens and operators combined, the **cobas**[®] EGFR Mutation Test had a correct call rate of 100% (108/108).

CLINICAL PERFORMANCE EVALUATION

Clinical Reproducibility

An external study was performed to assess the reproducibility of the **cobas**[®] EGFR Mutation Test across 3 external testing sites (2 operators per site), 3 reagent lots, and 5 non-consecutive testing days, with a 13-member panel of DNA samples extracted from FFPET sections of NSCLC Wild Type (WT) and Mutant type (MT) tumor specimens. This panel included the L858R mutation in exon 21 and five different exon 19 deletion mutations. Of 92 runs, 90 (97.8%) were valid. A total of 2,340 tests were performed on the 13 panel members in 90 valid runs; all test results were valid. There were no Mutation Detected results in 180 valid tests of WT panel members, producing 100% agreement. Agreements were 100% for 10 of the 12 MT panel members. For panel member EX19_2240_2257del18 – 5% Mutation, agreement was 62.8% (67 of 180 test results were Mutation not Detected). For panel member EX19_2240_2257del18 – 10% Mutation, agreement was 99.4% (1 of 180 test result was Mutation not Detected). Results by overall agreement are presented in Table 8 below. The coefficient of variation (CV) was <6% in all mutation panel members. Within each panel member, the CV was <3.5%. For external control the overall CV was <1.3%. The CV% was <0.5% for between lots and <1.2% for within-lot.

Table 8:
Overall Agreement Estimates by Panel Member in the **cobas[®] EGFR Mutation Test Reproducibility Study**

Panel Member	Number of Valid Tests	Agreement (N)	Agreement % (95% CI) ^a
Wild Type	180	180	100 (98.0, 100.0)
EX19_2235_2249del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2235_2249del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2236_2250del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2236_2250del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2239_2248>C - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2239_2248>C - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2254del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2254del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2257del18 - 5% Mutation	180	113	62.8 (55.3, 69.9)*
EX19_2240_2257del18 - ≤10% Mutation	180	179	99.4 (96.9, 100.0)*
EX21_2573T>G=L858R - 5% Mutation	180	180	100 (98.0, 100.0)
EX21_2573T>G=L858R - ≤10% Mutation	180	180	100 (98.0, 100.0)

Note: Results were in agreement when a Mutant Type panel member had a valid result of Mutation Detected or when Wild Type panel member had a valid result of Mutation Not Detected.

^a 95% CI = 95% exact binomial confidence interval.

* Analytical sensitivity of the **cobas**[®] EGFR Mutation Test for detecting this mutation is greater than 10% mutation level using the standard input of 50 ng per reaction well.

Correlation to Reference Method using Phase 3 Samples

The clinical performance of the **cobas**[®] EGFR Mutation Test was assessed by comparing it to two reference methods – 2x bidirectional Sanger sequencing and quantitative massively parallel sequencing (MPS) – using 487 formalin-fixed paraffin-embedded lung tumor specimens from patients with advanced NSCLC who were screened in the Phase 3 EURTAC trial of erlotinib vs. cisplatin-based chemotherapy.^{6,7} The clinical and demographic characteristics of the patients whose specimens were available for this retrospective testing were comparable to those of otherwise eligible patients (557) whose specimens were not available for retesting.

A total of 1,276 patients were screened for the EURTAC trial using a combination of laboratory developed tests, collectively referred to as the clinical trial assay (CTA). After excluding ineligible patients and those without CTA results, 1,044 patients were potentially eligible for the current study. Among the 1,044 eligible patients, 225 patients had samples that were mutation positive by CTA, 792 had samples that were Wild Type by CTA, and 27 had samples with inconclusive results by CTA. Of the 1,044 potentially eligible patients, 487 specimens were available for retesting with the **cobas**[®] EGFR Mutation Test.

All 487 specimens were tested in a blinded fashion with both the **cobas**[®] EGFR Mutation Test and Sanger sequencing. Of those, 406 had valid results by both the **cobas**[®] test and Sanger sequencing, 38 invalid results were observed by the **cobas**[®] test and Sanger sequencing, 38 invalid results by Sanger sequencing only, and 5 invalid results by the **cobas**[®] test only. Among the 487 specimens available for retesting with the **cobas**[®] EGFR Mutation Test, 444 specimens gave valid **cobas**[®] test results and were also tested with MPS. Of those, there were 36 invalid results by MPS; thus, 408 had valid results by both the **cobas**[®] test and MPS. The analytical accuracy of the **cobas**[®] EGFR Mutation Test compared with each reference method was evaluated by estimating the positive percentage agreement (PPA), negative percentage agreement (NPA), and overall percentage agreement (OPA) and their corresponding 95% CIs for exon 19 deletions and L858R mutations in aggregate, and separately.

In the EURTAC cohort, the **cobas**[®] EGFR Mutation Test detected the following deletion mutations in exon 19 of the EGFR gene: 2234_2251>AAT, 2235_2249del15, 2240_2254del15, 2238_2252del15, 2240_2257del18, 2236_2244del9, 2236_2263>GAAGCAT, 2236_2250del15, 2236_2252>AT, 2237_2255>T, 2237_2257>TCT, 2239_2248>C, 2239_2251>C, 2237_2251>AAC, 2239_2253>CAA, 2239_2256del18, 2239_2257>GT, and 2237_2253>TTGCT. The **cobas**[®] EGFR Mutation Test also detected the following L858R substitution mutation in exon 21 of the EGFR gene: 2573 T>G. Of the mutations detected in the EURTAC cohort, analytical sensitivity was demonstrated on the mutations listed in Table 3 above.

A total of 406 samples with valid **cobas**[®] test and Sanger results were included in the agreement analysis. The PPA between the **cobas**[®] EGFR Mutation Test and Sanger sequencing was 96.6% (95% CI: 91.5% to 98.7%), and the NPA was 88.3% (95% CI: 84.1% to 91.5%), in the detection of exon 19 deletions and L858R mutations in aggregate as presented in Table 9. The OPA was 90.6%, with the lower limit of the 95% CI above 87%. The PPA, NPA, and OPA in the detection of exon 19 deletion mutations were all >92%. The PPA, NPA, and OPA in the detection of L858R mutations compared were all >95%.

Table 9:

Comparison of the **cobas[®] EGFR Mutation Test with Sanger Sequencing for the Detection of EGFR Exon 19 Deletion Mutations and L858R Mutation**

cobas [®] EGFR Mutation Result	Sanger Sequencing Result								
	Result in aggregate			Exon 19 deletion			Exon 21 Mutation		
	MD	MND	Total	MD	MND	Total	MD	MND	Total
MD	112	34	146	71	25	96	41	9	50
MND	4	256	260	2	308	310	2	354	356
Total	116	290	406	73	333	406	43	363	406
PPA (95% CI)	112/116 = 96.6% (91.5%, 98.7%)			71/73 = 97.3% (90.5%, 99.2%)			41/43 = 95.3% (84.5%, 98.7%)		
NPA (95% CI)	256/290 = 88.3% (84.1%, 91.5%)			308/333 = 92.5% (89.2%, 94.9%)			354/363 = 97.5% (95.4%, 98.7%)		
OPA (95% CI)	368/406 = 90.6% (87.4%, 93.1%)			379/406 = 93.3% (90.5%, 95.4%)			395/406 = 97.3% (95.2%, 98.5%)		

A total of 408 samples with valid **cobas**[®] test and MPS results were included in the agreement analysis. By comparison, the PPA and NPA between the **cobas**[®] EGFR Mutation Test and MPS for the detection of exon 19 deletions and the L858R point mutation in aggregate were 94.0% (95% CI: 89.1% to 96.8%) and 97.7% (95% CI: 95.0% to 98.9%), respectively as presented in Table 10. The OPA was 96.3%, with a lower limit of the 95% CI of 94.0%. The PPA, NPA, and OPA in detecting exon 19 deletion mutations were all >95%, with all the 95% lower limit CIs >90%. The PPA, NPA, and OPA in detecting the L858R mutation were also all >95%, with all lower limits of the 95% CIs ≥ 95% except for PPA (90%), owing to due to the small number of L858R mutation detected.

Table 10:

Comparison of the cobas[®] EGFR Mutation Test with MPS for the Detection of EGFR Exon 19 Deletion Mutations and L858R Mutation

cobas [®] EGFR Mutation Result	MPS Result								
	Result in aggregate			Exon 19 deletion			Exon 21 Mutation		
	MD	MND	Total	MD	MND	Total	MD	MND	Total
MD	142	6	148	94	1	95	48	5	53
MND	9	251	260	4	309	313	5	350	355
Total	151	257	408	98	310	408	53	355	408
PPA (95% CI)	142/151 = 94.0% (89.1%, 96.8%)			94/98 = 95.9% (90.0%, 98.4%)			48/53 = 90.6% (79.7%, 95.9%)		
NPA (95% CI)	251/257 = 97.7% (95.0%, 98.9%)			309/310 = 99.7% (98.2%, 99.9%)			350/355 = 98.6% (96.7%, 99.4%)		
OPA (95% CI)	393/408 = 96.3% (94.0%, 97.8%)			403/408 = 98.8% (97.2%, 99.5%)			398/408 = 97.5% (95.5%, 98.7%)		

Clinical Outcome Data

The EURTAC trial was a phase 3, multicenter, open-label, randomized study of Tarceva[®] (erlotinib) versus standard platinum doublet chemotherapy as first-line therapy in chemotherapy-naïve patients with advanced NSCLC whose tumors harbor EGFR exon 19 deletions or exon 21 (L858R) substitution mutations, as assessed by a clinical trial assay (CTA). The study was conducted under the sponsorship of the Spanish Lung Cancer Group (SLCG). A total number of 174 patients were enrolled into the study. The trial results showed that patients who received Tarceva[®] had a statistically significant increase in progression-free survival (PFS) (median PFS 10.4 months vs. 5.1 months) as compared to patients who received chemotherapy, with a hazard ratio of 0.34 (p < 0.0001, 95% CI [0.23; 0.49]). The response rate of patients on the Tarceva[®] arm was greater than the response rate of patients treated with chemotherapy (65.1% vs. 16.1%). No significant difference was observed in overall survival (OS) in the two arms, as 76% of patients on the standard chemotherapy arm crossed over to receive Tarceva[®].

Of the 174 patients enrolled into the EURTAC trial, 134 cases (77% of the study population, including 69 patients from the erlotinib arm and 65 patients from the chemotherapy arm) were available for retesting and tested retrospectively by the cobas[®] EGFR Mutation Test. Of the 134 cobas[®] test retested cases, 116 cases (59 patients from the erlotinib arm and 57 patients from the chemotherapy arm) were “Mutation Detected” by the cobas[®] EGFR Mutation Test. Analysis of the 116 subset revealed that those patients treated with Tarceva had a significant increase in PFS time (median PFS 10.4 vs. 5.4 months and less likely to have an event of progressive disease or death (HR= 0.34, 95 % CI [0.21;0.54], p<0.0001) than patients treated with chemotherapy (Figure 2). The response rate in the Tarceva arm was greater compared to the chemotherapy arm (59.3% vs. 14.0%). No significant difference in OS was observed between the two groups. The observed clinical benefit in the subset of patients tested with the cobas[®] EGFR Mutation Test was comparable to that observed in the full study population (Table 11 below).

Additional efficacy analysis was conducted to consider patients who were tested positive by the cobas[®] EGFR Mutation Test but were tested negative or invalid by the CTA. In the worst case scenario (assuming a hazard ratio of 1 for patients positive by the cobas[®] test and negative by CTA), data demonstrated a hazard ratio of 0.42 (95 % CI [0.26; 0.57]).

Figure 2:
Kaplan-Meier Plot of PFS by Treatment for Patients with Mutation Detected by the cobas[®] EGFR Mutation Test (Investigator Assessment)

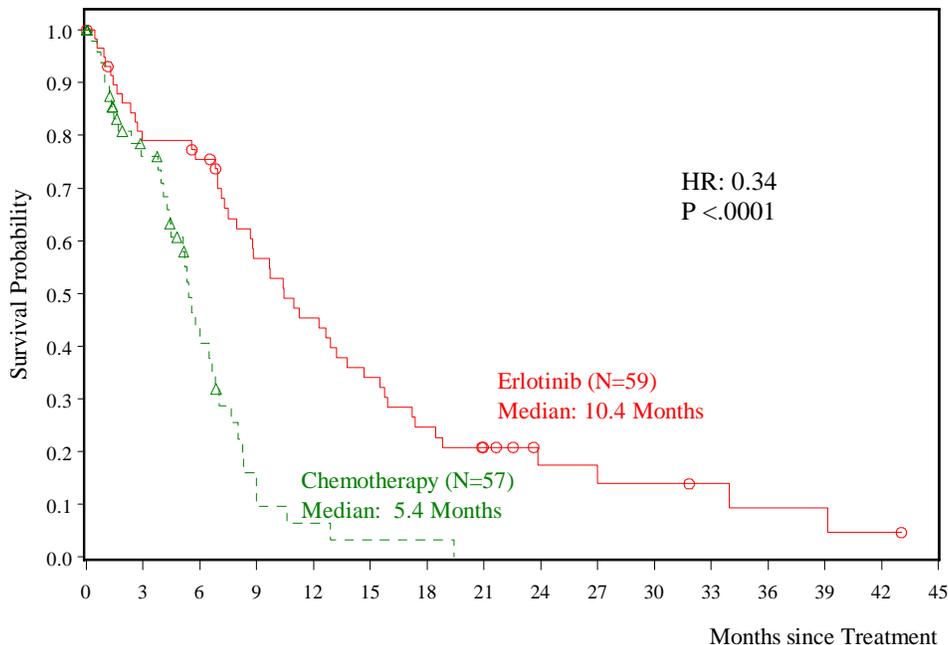


Table 11: Clinical Benefit of Patients Tested With The cobas[®] EGFR Mutation Test is Comparable to That Observed in the EURTAC Population

Parameter	cobas [®] -positive Population n = 116		EURTAC n = 173*	
	Chemotherapy n = 57	Erlotinib n = 59	Chemotherapy n = 87	Erlotinib n = 86
PFS				
Median (Months)	5.4	10.4	5.1	10.4
Hazard Ratio	0.34		0.34	
Hazard Ratio 95% CI	[0.21; 0.54]		[0.23; 0.49]	
P-Value (log-rank test)	<0.0001		<0.0001	

*Note: One patient withdrew consent after completion of the EURTAC study, which resulted in a dataset of n = 173

REFERENCES

- Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nature Reviews Cancer*. 2007;7:169-181.
- Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer*. 2010;10:760-774.
- Zhou C, Wu Y-L, Chen G, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Onc*. 2011; 12:735-742
- Paz-Ares L, Soulières D, Melezínek I, et al. Clinical outcomes in non-small-cell lung cancer patients with EGFR mutations: pooled analysis. *J Cell Mol Med* 2010;14:51-69.
- American Cancer Society. *Cancer Facts and Figures*, 2012.
- Tarceva[®] (erlotinib) Package Insert
- Rosell, R., et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *The Lancet Oncology*. 2012;13 v3:239-246.
- Longo, M.C., Berninger, M.S. and Hartley, J.L. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*. 93:125-128.
- Chosewood, L.C. and Wilson, D.E. *Biosafety and Microbiological and Biomedical Laboratories*. HHS Publication Fifth # edition.(CDC) 21-1112. 2009.
- Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers from Occupationally Acquired Infections. Approved Guideline-Third Edition*. CLSI Document M29-A3 Villanova, PA:CLSI, 2005.
- International Air Transport Association. *Dangerous Goods Regulations*, 52nd Edition. 2011.
- Costa DB, Nguyen KS, Cho BC, Sequist LV, Jackman DM, Riely GJ, Yeap BY, Halmos B, Kim JH, Jänne PA, Huberman MS, Pao W, Tenen DG, Kobayashi S. Effects of erlotinib in EGFR mutated non-small cell lung cancers with resistance to gefitinib. *Clin Cancer Res*. 2008 Nov 1;14(21):7060-7
- Catalogue of Somatic Mutations in Cancer (COSMIC), 2011, v.51, <http://www.sanger.ac.uk/genetics/CGP/cosmic>.
- Clinical and Laboratory Standards Institute (CLSI) EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guidelines – Second Edition, Appendix D* 2005.

Document Revision Information

Doc Rev. 1.0
4/2013

First Publishing.



Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
A Member of the Roche Group



Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the Roche Response

Distributed by Center toll free: 1-800-526-1247)

COBAS, COBAS Z, and AMPERASE are trademarks of Roche.

Carryover prevention technology in the AmpErase enzyme is covered by U.S. Patent 5,035,996 and foreign counterparts owned by Invitrogen Corporation and licensed to Roche Molecular Systems, Inc.

The cyanine dyes in this product are subject to patent rights of GE Healthcare Bio-Sciences Corp. and Carnegie Mellon University and are licensed to Roche solely for incorporation into components of research and in-vitro diagnostic kits. Any use of such kits for purposes other than research or in-vitro diagnostics requires sublicenses from GE Healthcare Bio-Sciences Corp., Piscataway, New Jersey, U.S.A. and Carnegie Mellon University, Pittsburgh, Pennsylvania, U.S.A.

Certain EGFR sequences in this product are covered by one of more patents of Genzyme Corp. and Dana Farber Cancer Institute and The General Hospital Corporation and licensed to Roche Molecular Systems, Inc. under U.S. Patent No. 7,964,349 and other U.S. and foreign patents pending.

EPPENDORF is a trademark of Eppendorf AG.

PIPET-AID is a trademark of Drummond Scientific.

NANODROP is a trademark of Thermo Scientific.

Copyright 2013 Roche Molecular Systems, Inc.

07/2013

Doc Rev. 2.0

06356575001-02

The following symbols are now used in labeling for Roche PCR diagnostic products.



Ancillary Software



Batch code



Authorized representative in the European Community



Biological Risk (Potentially biohazardous material)



Barcode Data Sheet



Catalogue Number



Consult instructions for use



For IVD Performance Evaluation only



Contains sufficient for < n > tests



Lower Limit of Assigned Range



Contents of kit



Manufacturer



Distributed by



Store in the dark



In vitro diagnostics medical device



Temperature limitation (Store At)



Test Definition File



Use by (last day of month)



Upper Limit of Assigned Range

US Customer Technical Support 1-800-428-2336