

PRO-Trac™ II Tacrolimus ELISA Kit Part 32400
P970025

Name of Label	Label Part Number
Kit Contents Label	32400
Microtiter Plate	26148
Chromogen	26151
Stop Solution	26155
10X Wash Solution	24192
Control 1	26156
Control 2	26157
Zero (0) Standard	24193
A Standard	24194
B Standard	24195
C Standard	24196
D Standard	24197
E Standard	24198
Digestion Reagent	26162
Conjugate Diluent	26160
Antibody	26158
5X Conjugate	26159
Product Instruction Manual	15738

KIT CONTENTS LABEL for
PRO-Trac™ II Tacrolimus ELISA Kit Part 32400
P970025

PRO-Trac™ II
Tacrolimus ELISA Kit

Cat.#/Ref./KAT.NR. 32400
96 Wells/Puits/
Vertiefungen

FOR IN VITRO DIAGNOSTIC USE. Not for internal or external use in humans or animals.
Contains instructions and materials for the quantitative determination of Tacrolimus (Prograf®, FK 506)
in human EDTA whole blood.
POUR DIAGNOSTIC IN VITRO. Fourni avec instructions et matériel pour la détermination quantitative
de Tacrolimus (Prograf®, FK 506) dans le sang total humain contenant de l'EDTA.
NUR ZUR IN VITRO DIAGNOSTIK. Enthält Anweisungen und Material zur quantitativen Bestimmung von
Tacrolimus (Prograf®, FK 506) in menschlichem EDTA-Vollblut.

VIALS (EACH) LYOPHILIZED, mL after reconstitution: (1) Digestion Reagent, 20 mL.
VIALS (EACH) LIQUID, mL: (1) Antibody, 8 mL; (1) SX Conjugate, 2.0 mL; (1) O Standard, 2.0 mL;
(5) Standards (A-E), 0.5 mL; (2) Controls, 0.5 mL; (1) Chromogen, 22 mL; (1) Stop Solution, 15 mL;
(1) 10X Wash Solution, 50 mL; (1) Conjugate Diluent, 9 mL. **OTHER:** (1) Microtiter Plate; (1) Plate Cover.
FLACONS (CHAQUE) LYOPHILISÉ, ÉQUI. à X mL: (1) Réactif de digestion, 20 mL.
FLACONS (CHAQUE) EN SOLUTION, mL: (1) Anticorps, 8 mL; (1) SX Conjugué, 2.0 mL; (1) d'étalon zéro, 2.0 mL;
(5) Étalons (A-E), 0,5 mL; (2) Contrôles, 0,5 mL; (1) Chromogène, 22 mL; (1) Solution d'arrêt, 15 mL;
(1) 10X Solution de lavage, 50 mL; (1) Diluant conjugué, 9 mL. **PLUS:** (1) Microplaque; (1) Couvre-plaque.
FLÄSCHCHEN (ANZAHL) LYOPHILISIERT, mL nach Auflösen: (1) Verdauungsreagens, 20 mL.
FLÄSCHCHEN (ANZAHL) GEBRAUCHSFERTIG, mL: (1) Antikörper, 8 mL; (1) SX Konjugat, 2,0 mL; (1) Nullstandard, 2,0 mL;
(5) Standards (A-E), 0,5 mL; (2) Kontrollen, 0,5 mL; (1) Chromogen, 22 mL; (1) Stopplösung, 15 mL;
(1) 10X Waschlösung, 50 mL; (1) Konjugate Verdünnungslösung, 9 mL. **ZUSÄTZLICH:** (1) Mikrotitrierplatte; (1) Abdeckplatte.

Upon receipt store standards, controls, SX conjugate, and digestion reagent at/Dès réception, stocker les étalons,
contrôles, conjugué SX et réactif de digestion entre/Nach Empfang sind die Standards, Kontrollen, SX-Konjugat und
Auffarblösungsreagenzien zu lagern bei: -18 to -25°C
Store all other reagents at/Stockez tous les autres réactifs entre/Alle anderen Reagenzien lagern bei: 2-8°C

* 2N H₂SO₄ (R 34, S 26)
R 34 - Causes burns/Provoque des brûlures./Verursacht Verätzungen./Provoca ustión.
Provoca quemaduras. S 26 - In case of contact with eyes, rinse immediately with plenty
of water and seek medical advice./En cas de contact avec les yeux, laver immédiatement
et abondamment avec de l'eau et consulter un spécialiste./Bei Berührung mit den Augen
gründlich mit Wasser abspülen und Arzt konsultieren. /In caso di contatto con gli occhi,
lavare immediatamente e abbondantemente con acqua e consultare un medico. /En
caso de contacto con los ojos, lavarse inmediata y abundantemente con agua
y acudir a un médico.

Enreg. AFSSAPS N° R53992

Contains human source material. Handle as potentially infectious.
Contient des substances d'origine humaine. Manipuler comme tout
produit potentiellement infectieux. /Enthält Material humanen Ursprungs,
daher wie potentiellen Infektionserreger behandeln.

Lot/Ch.-B.:

Exp./Verw.bis:



C
Cemester
Cemest
Azend/
Cemstvo

DiaSorin Inc., Stillwater, MN 55082 U.S.A. (651) 439-6710
DiaSorin S.p.A., 11 rue Georges Besse, 92160 Antony, France 01 35 99 04 00

22407

VIAL LABELS for PRO-Trac™ II Tacrolimus ELISA Kit Part 32400 P970025

**PRO-Trac™ II Tacrolimus
Microtiter Plate** 26148
Microplaque/Mikrotiterplatte 12 x 8 Wells/
Puits/
Vertiefungen

For In Vitro Diagnostic Use / In Vitro Test.

Store/Cons./Lag.: 2-8°C

Lot/Ch. #:
Exp./Valid. bis:
DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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Chromogen 26151
Chromogène

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 22 mL
Store/Cons./Lag.: 2-8°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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Stop Solution 26155
Solution d'arrêt

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/
Gebrauchsfertig: 15 mL
Store/Cons./Lag.: 2-8°C

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DiaSorin S.A., 92180 Antony, France

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10X Wash Solution 24192
Solution de lavage X10

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 50 mL
Store/Cons./Lag.: 2-8°C

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DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 26156
Control/Contrôle/Kontrolle
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

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PRO-Trac™ II Tacrolimus 26157
Control/Contrôle/Kontrolle
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
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PRO-Trac™ II Tacrolimus 24193
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 2.0 (2,0) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 24194
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 24195
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 24196
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 24197
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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PRO-Trac™ II Tacrolimus 24198
Standard/Etalon
(Human/Humain)

For In Vitro Diagnostic Use / In Vitro Test.
Liquid/Solution/Gebrauchsfertig: 0.5 (0,5) mL
Store/Cons./Lag.: -18 to -25°C

DiaSorin Inc., Stillwater, MN 55082 U.S.A.
DiaSorin S.A., 92180 Antony, France

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VIAL LABELS for
PRO-Trac™ II Tacrolimus ELISA Kit Part 32400
P970025

PRO-Trac™ II Tacrolimus
Digestion Reagent **26162**
Réactif de digestion/Verdauungsreagens

For In Vitro Diagnostic Use./In Vitro Test. (Lyoph.)
 After recons./Après reconst./Nach Ansetzen **20 mL**
Store/Cons./Lag.: -18 to -25°C

DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

Lot Ch. B.
 Exp. Date 03

PRO-Trac™ II Tacrolimus
Conjugate Diluent **26160**
Diluant Conj./Konj. Verdünn.

For In Vitro Diagnostic Use./In Vitro Test
 Liquid/Solution/Gebrauchsfertig: **9 mL**
Store/Cons./Lag.: 2-8°C
 DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

Lot Ch. B.
 Exp. Date 03

PRO-Trac™ II Tacrolimus
Antibody/Anticorps **26158**

For In Vitro Diagnostic Use./In Vitro Test.
 Liquid/Solution/Gebrauchsfertig: **8 mL**
Store/Cons./Lag.: 2-8°C
 DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

Lot Ch. B.
 Exp. Date 03

PRO-Trac™ II Tacrolimus
5X Conjugate/5X Conjugat **26159**
5X Konjugat

For In Vitro Diagnostic Use./In Vitro Test
 Liquid/Solution/Gebrauchsfertig: **2.0 (2.0) mL**
Store/Cons./Lag.: -18 to -25°C
 DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

DaSern Inc., Stillwater, MN 55082 U.S.A.
 DaSern S.A., 92180 Antony, France

Lot Ch. B.
 Exp. Date 03

**Product Instruction Manual for
PRO-Trac™ II Tacrolimus ELISA Kit Part 32400
P970025**

See attached.

**PRO-Trac™ II Tacrolimus
ELISA Kit**

For the quantitative determination of Tacrolimus
(Prograf®, FK506) in human EDTA or heparinized whole blood
U.S. Patent #5,650,288

Instruction Manual

Catalog No: 32400

DiaSorin

Stillwater, Minnesota 55082-0285, U.S.A.

TABLE OF CONTENTS

Introduction.....	Page 1
Reagents	2
Warnings & Precautions.....	3
Specimen Requirements	4
Procedure.....	5
Quality Control.....	7
Results.....	7
Expected Values.....	9
Performance Data	10

INTRODUCTION

Intended Use

The PRO-Trac™ II Tacrolimus ELISA is an *in vitro* reagent system intended for the quantitative determination of tacrolimus (Prograf®, FK506) and some metabolites in human EDTA or heparinized whole blood as an aid in the management of liver transplant patients receiving tacrolimus therapy.

CAUTION: U.S. FEDERAL LAW RESTRICTS THIS DEVICE TO SALE AND DISTRIBUTION BY, OR ON THE ORDER OF, A PHYSICIAN, OR TO A CLINICAL LABORATORY; AND USE IS RESTRICTED TO, BY, OR ON THE ORDER OF, A PHYSICIAN. THE PRO-TRAC™ II Tacrolimus ELISA KIT IS FOR USE ONLY WITH TACROLIMUS.

Warnings: No firmly established therapeutic range exists for effective tacrolimus concentration in whole blood. Absorption and clearance of tacrolimus can vary greatly among patients. Clinical response to tacrolimus treatment does not correlate well with the administered dose. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic and toxic effects of tacrolimus, coadministration of other immunosuppressants, time post transplant, and a number of other factors will result in different requirements for optimal blood levels of tacrolimus. Individual tacrolimus values can not be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made.

Precautions: Precautions for use of the device are found in "Warnings and Precautions".

Summary and Explanation

Tacrolimus (Prograf®[®], FK506) is a macrolide lactone of fungal origin with strong immunosuppressive properties.¹ It has been shown to have immunosuppressive effects *in vitro* through the inhibition of mixed lymphocyte reactivity and the generation of cytotoxic T cells.^{2,3} It also acts by suppressing the production of cytokines, including IL-2; suppressing the expression of IL-2 receptors on activated T cells. Tacrolimus has an *in vitro* potency 50-100 times greater than Cyclosporin A.^{1,2} The drug is bound to an abundant cytosolic protein which has peptidyl-prolyl *cis-trans* isomerase activity. This tacrolimus binding protein, which is distinct from the cyclophilin which binds Cyclosporin A, is an enzyme that is partly responsible for the folding of proteins and peptides into their native conformation.^{3,4} As with Cyclosporin A, tacrolimus is mostly bound within erythrocytes which contain large amounts of the specific binding protein. When the drug is released from the cells, it is metabolized extensively by the liver with less than 5% excreted in urine. Greater than 95% of the drug is eliminated by the biliary route, mainly as drug metabolites.^{3,4}

Tacrolimus is used to prevent organ rejection in liver transplant patients. It is also being used in experimental trials for kidney, heart, and bone marrow transplant patients. Despite its therapeutic properties, tacrolimus exhibits some toxicity. Its adverse effects resemble those of Cyclosporin A and include nephrotoxicity, gastrointestinal tract complaints, neurotoxicity, and glucose intolerance.⁵ The importance of therapeutic monitoring of tacrolimus concentrations relates to the poor correlation of dose to blood concentration, the moderate variability of pharmacokinetic parameters between patients, and the narrow therapeutic window.⁶ Due to the observed inter-patient variability, a therapeutic range for tacrolimus blood levels in liver transplant patients can not be established from current clinical studies.

Five general methods exist for the measurement of tacrolimus in whole blood: receptor binding,⁷ bioassay,⁷ high pressure liquid chromatography (HPLC) with various detection methods,^{8,9} microparticle enzyme immunoassay (MEIA),¹⁰ and enzyme-linked immunosorbent assay (ELISA)¹¹⁻¹³ technologies. Due to the differences in methodologies, extraction procedures, and metabolite recognition, results are not interchangeable

between all methods. Unlike receptor binding, bioassay and HPLC methods which are restricted in use; current immunoassays are practical for routine clinical use and allow for rapid analysis of tacrolimus in whole blood.

Method Description

The DiaSorin PRO-Trac™ II Tacrolimus ELISA is a sensitive competitive enzyme immunoassay which employs a monoclonal antibody to tacrolimus. This assay is run in a microtiter plate which has been precoated with goat anti-mouse IgG. Standards, controls, and samples are extracted with a proprietary reagent (U.S. Patent #5,650,288), then added to the wells of the microtiter plate followed by addition of the anti-tacrolimus monoclonal antibody. After a thirty minute incubation at room temperature, the tacrolimus-horseradish peroxidase conjugate is added and incubated an additional sixty minutes. The wells are then washed and chromogen is added for a fifteen minute incubation. This reaction is stopped by addition of acid and the absorbance in each well is read at a dual wavelength of 450/630 nm. Color development is inversely proportional to the amount of tacrolimus present in the sample. Concentrations are read from a standard curve.

REAGENTS

Cat. No. 32400 sufficient for 39 determinations in duplicate.

Upon receipt, store the microtiter plate, antibody, wash concentrate, conjugate diluent, stop solution, and chromogen at 2 to 8°C. Store the concentrated conjugate, standards, controls, and digestion reagent at -18 to -25°C. Unopened components are stable at recommended temperatures until labeled expiration date.

1. PRO-Trac™ II Tacrolimus Standards. Part Numbers 24193 - 24198.

Standards consist of purified tacrolimus in a processed human whole blood matrix with preservatives (ProClin™ 150). Standards are provided ready to use at six nominal concentrations (0, 0.3, 1.0, 3.0, 10.0, and 30.0 ng/mL) and should be stored at -18 to -25°C. Up to three freeze-thaw cycles are permitted for these reagents.

2. PRO-Trac™ II Tacrolimus Controls, Levels 1 and 2. Part Numbers 26156 - 26157.

Controls consist of purified tacrolimus (FK506) in a processed human whole blood matrix with preservatives (ProClin™ 150). Controls are provided ready to use at two nominal concentrations (2.0 and 15.0 ng/mL) and should be stored at -18 to -25°C. Up to three freeze-thaw cycles are permitted for these reagents.

3. PRO-Trac™ II Tacrolimus Microtiter Plate. Part Number 26148.

The microtiter plate provided with the kit is coated with goat anti-mouse IgG. The plate is provided ready to use and should be stored at 2 to 8°C.

4. PRO-Trac™ II Tacrolimus 5X Conjugate. Part Number 26159.

The 5X conjugate consists of a 5X solution of tacrolimus-horseradish peroxidase in Tris buffer with enzyme stabilizers and preservatives (ProClin™ 300). The 5X conjugate should be stored at -18 to -25°C. Up to three freeze-thaw cycles are permitted for this reagent. Protect from light. Dilute prior to use.

5. PRO-Trac™ II Tacrolimus Conjugate Diluent. Part Number 26160.

The conjugate diluent consist of a Tris buffer with bovine serum albumin and preservatives (ProClin™ 300). The conjugate diluent is provided ready to use and should be stored at 2 to 8°C.

6. PRO-Trac™ II Tacrolimus Antibody. Part Number 26158.

The monoclonal antibody consists of a murine anti-tacrolimus antibody in a Tris buffer with bovine serum albumin and preservatives (ProClin™ 300). The monoclonal antibody is provided ready to use and should be stored at 2 to 8°C.

7. PRO-Trac™ II Tacrolimus Digestion Reagent. Part Number 26162.

The digestion reagent consists of a lyophilized mixture of bacterial Proteinase K and Subtilisin in Tris buffer containing saponin. The digestion reagent must be reconstituted with distilled or deionized water prior to use and should be stored at -18 to -25°C. When reconstituted, the total proteolytic activity is 2.0 U/mL.

8. 10X Wash Solution. Part Number 24192.

The wash solution consists of a 10X solution of phosphate buffer, Tween 80, and ProClin™ 300. The wash buffer must be diluted prior to use with distilled or deionized water, and should be stored at 2 to 8°C.

9. Stop Solution. Part Number 26155.

The stop solution consists of a solution of 2 N sulfuric acid (H₂SO₄). The stop solution is provided ready to use and should be stored at 2 to 8°C.

10. PRO-Trac™ II Tacrolimus Chromogen. Part Number 26151.

The chromogen is a buffered substrate and chromogen [3,3',5,5'-tetramethylbenzidine (TMB)]. The chromogen is provided ready to use and should be stored at 2 to 8°C, protected from light.

11. Microtiter Plate Cover.

A plastic plate cover is provided to cover the plate during incubations.

12. Product Insert/Instruction Manual.

Complete instructions, representative performance data, and references are contained in the product insert.

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE.

Not for internal or external use in humans or animals.

Reagents Containing Human Source Material

Treat as potentially infectious.

Each serum/plasma donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HBsAg, antibody to HCV and antibody to HIV. While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source material for which there is no approved test. Because no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus (HCV), Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all products containing human source material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 3rd ed., 1993. HHS Publication No. (CDC) 93-8395.

Reagents Containing > 0.4% ProClin™ 150

May cause allergic reactions. Avoid prolonged contact with skin. Wash thoroughly after handling.

Reagents Containing 3,3',5,5'-Tetramethylbenzidine

This product contains 3,3',5,5'-tetramethylbenzidine (TMB) [$\leq 0.05\%$] which has shown possible mutagenic effects in laboratory experiments.

Reagents Containing H₂SO₄

This product contains 2 N H₂SO₄ (Stop Solution): Danger! Causes severe burns. Do not get in eyes, on skin, or on clothing. In case of contact, immediately flush eyes and skin with plenty of water.

ProClin is a registered trademark of Rohm and Haas Company.

European Community Hazardous Substance Risk Phrases (Council Directive 88/379/EEC)

R34 - Causes Burns

S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Indications of Possible Deterioration of Kit Reagents

1. The presence of abnormal particulate matter in any of the reagents.
2. A shift in the slope or position of the standard curve from what is normally obtained.
3. A maximum Zero Standard absorbance < 1.50 Absorbance Units (AU).
4. A high non-specific binding.
5. Poor reproducibility between duplicates.
6. Control values out of range.

SPECIMEN REQUIREMENTS

Collection, Preparation, and Storage of EDTA or Heparinized Whole Blood

Fifty microliters of whole blood anticoagulated with either EDTA or heparin are required for the PRO-Trac™ II Tacrolimus ELISA procedure. Collect blood by venipuncture in a 5 or 10 mL evacuated glass tube. No further additives or preservatives are required to maintain the integrity of the sample. If the specimens are not used immediately, store at -18 to -25°C for up to 6 months.^{4,5,6} Fresh samples that have not been immediately processed, or whole blood samples that have been stored at -18 to -25°C, must be thoroughly mixed prior to taking samples for testing in this kit. Specimens should be subject to ≤ 3 freeze/thaw cycles.

At high tacrolimus levels (>20 ng/mL), differences of 0.7 – 5.9 ng/mL were observed in tacrolimus values between whole blood anticoagulated with heparin and whole blood anticoagulated with EDTA.

Sample stability was examined to support shipment of samples to regional testing facilities. Fresh samples (assayed within 72 hrs) were compared with frozen samples by linear regression and shown to be equivalent ($r = 0.91$). Clinical samples containing tacrolimus have been reported to be stable at room temperature for up to 14 days,^{4,5,6} and frozen for 6 months.¹

In clinical studies conducted by DiaSorin,

Patient specimens collected in EDTA tubes and assayed within 72 hours of collection at room temperature exhibited mean (\pm SD) percentage recovery relative to assay by HPLC/MS/MS of $117\% \pm 14\%$ (range 96% - 136%), tacrolimus range 1.4 – 24.5 ng/mL.

Patient specimens collected in EDTA tubes may be stored up to 7 days at room temperature prior to testing. The mean (\pm SD) absolute difference from samples assayed within 24 hours of collection is $8\% \pm 3\%$ (range 2% - 14%), tacrolimus range 5.0 – 21.7 ng/mL.

Patient specimens collected in EDTA may be stored frozen at -20°C prior to assay. The mean (\pm SD) absolute difference between fresh samples stored at room temperature, assayed within 24 hours of collection and samples stored at -20°C for 3 – 14 days is $16\% \pm 10\%$ (range 0% - 49%), tacrolimus range 0.0 – 15.3 ng/mL.

Patient specimens collected in heparinized tubes and assayed within 72 of collection hours at room temperature exhibited a mean (\pm SD) absolute difference from EDTA samples of $6\% \pm 6\%$ (range 1% - 24%), tacrolimus range 2.8 – 23.8 ng/mL.

PROCEDURE

Reagents Supplied with Each Kit

	Cat. No. 32400
1. PRO-Trac™ II TACROLIMUS MICROTITER PLATE	1 plate 96 wells
2. PRO-Trac™ II TACROLIMUS ANTIBODY	1 vial 8 mL
3. PRO-Trac™ II TACROLIMUS 5X CONJUGATE	1 vial 2 mL
4. PRO-Trac™ II TACROLIMUS CONJUGATE DILUENT	1 vial 9 mL
5. PRO-Trac™ II TACROLIMUS STANDARDS	5 vial 0.5 mL
6. PRO-Trac™ II TACROLIMUS ZERO STANDARD	1 vial 2 mL
7. PRO-Trac™ II TACROLIMUS CONTROLS	2 vials 0.5 mL
8. PRO-Trac™ II TACROLIMUS CHROMOGEN	1 vial 22 mL
9. STOP SOLUTION (2N H ₂ SO ₄)	1 vial 15 mL
10. 10X WASH SOLUTION	1 vial 50 mL
11. PRO-Trac™ II TACROLIMUS DIGESTION REAGENT	1 vial Lyophilized
12. PLATE COVER	1 cover

Materials Required But Not Supplied

In addition to the reagents supplied with the DiaSorin PRO-Trac™ II Tacrolimus kit, the following are required:

1. Manual or automated microtiter plate washer.
2. Microtiter plate reader, 450/630 nm reading capability (the DiaSorin QC laboratory uses either the BioTek EL312a or Elx800).
3. Data reduction capable of generating a 4 Parameter Logistics (4PL) curve fit (the DiaSorin QC laboratory uses the MultiCalc™ data reduction program by Wallac).
4. Centrifuge capable of 1800 x g*.
5. 12 x 75 conical bottom polypropylene tubes.
6. Water bath capable of 75°C.
7. Adjustable micropipettors with disposable tips (25, 50, 100, 200, 1000 µL capability) or calibrated pipets (50 and 100 µL capability).
8. Microtiter orbital plate shaker capable of operating at 700 rpm.

$$*g = (1118 \times 10^3) (\text{radius in cm}) (\text{rpm})^2$$

9. Reagent reservoirs.
10. Adjustable multichannel pipettors with disposable tips (200 μ L capability).

REFER TO BACK PAGE FOR PLATE MAP

Assay Procedure

1. Bring all reagents to room temperature. Turn on water bath and set temperature to 75°C.
2. Dilute the 10X Wash Solution 1:10 with deionized or distilled water.
3. Label one 12 x 75 mm conical bottom tube for each standard, NSB, control and sample. Glass 12 x 75 mm tubes may be used, although conical bottom tubes are recommended. Do not mix glass and plastic tubes within a single assay.
4. Pipette 50 μ L of each sample into the appropriately labeled tube. A second zero standard sample should be extracted to be used as the NSB.
5. Reconstitute Digestion Reagent with 20 mL of room temperature (20 - 25°C) deionized or distilled water. Allow to stand at room temperature for 10 minutes. Mix thoroughly. Use within 30 minutes. Freeze (-20°C) any unused portion immediately. Reconstituted Digestion Reagent is stable at -20°C for at least 6 months. Three freeze thaw cycles are permitted.
6. Pipette 300 μ L of reconstituted Digestion Reagent into each tube. Cover the tubes with foil (or cap tubes). Vortex all tubes 15 - 30 seconds.
7. Incubate at room temperature (20 - 25°C) for 15 \pm 2 minutes.
8. Transfer tubes to 75 \pm 1°C water bath and incubate for 15 \pm 2 minutes.
9. Remove tubes from water bath and vortex for 15 - 30 seconds.
10. Centrifuge tubes for 10 minutes at 1800 x g at room temperature (20 - 25°C).
11. Pipette 100 μ L of each samples' supernatant into duplicate wells in the Microtiter Plate, taking care not to disturb the pellet.
12. Pipette 50 μ L of Anti-Tacrolimus Monoclonal Antibody to all wells except the NSB wells. Pipette 50 μ L of Conjugate Diluent into the NSB wells.
13. Cover the wells with parafilm and a plate cover and fasten the unit to a plate shaker. Shake the plate for 30 \pm 2 minutes at 700 \pm 50 rpm and room temperature (20 - 25°C).
14. Dilute the 5x Conjugate to working strength (1x) with Conjugate Diluent (i.e. 1 mL Conjugate Concentrate + 4 mL Conjugate Diluent). Prepare enough working strength conjugate for that day's assay. Excess diluted conjugate is discarded.
15. Pipette 50 μ L of the diluted conjugate to each well.
16. Cover the wells with parafilm and a plate cover and return the unit to a plate shaker. Shake the plate for 60 \pm 5 minutes at 700 \pm 50 rpm and room temperature (20 - 25°C).
17. Wash each well three times with approximately 300 μ L of diluted Wash Solution. Shake any residual Wash Solution from the wells by inverting the plate and pounding against absorbent paper.
18. Pipette 200 μ L of Chromogen to each well within 5 minutes of washing the plate. Cover the wells with parafilm and a plate cover and shake for 15 \pm 1 minute at room temperature (20 - 25°C) and 700 \pm 50 rpm.
19. Vigorously pipette 100 μ L of Stop Solution to the wells. The color changes from blue to yellow. The Stop Solution should be added with enough force to mix the wells thoroughly in order to completely stop the reaction.
20. Read the plate on a microtiter plate reader at 450/630 nm dual wavelengths. The plate should be read within 5 minutes of stopping the reaction.

$$*g = (1118 \times 10^4) (\text{radius in cm}) (\text{rpm})^2$$

- 21 Use a 4PL curve fitting program, plotting absorbance vs log concentration, to interpolate the concentrations of the controls and samples from the standard curve.

Procedural Comments

1. The absorbance of the NSB should be less than 0.200 absorbance units.
2. Protect the PRO-Trac™ II Tacrolimus Chromogen and the PRO-Trac™ II Tacrolimus SX Conjugate from direct light.
3. The PRO-Trac™ II Tacrolimus SX Conjugate is a viscous solution which must be pipetted with care to ensure accurate results. No special precautions are needed for the working strength conjugate solution.
4. To convert ng/mL to mol/L, multiply the ng/mL by the following conversion: 1.13×10^{-6} .

Standardization

The DiaSorin PRO-Trac™ II Tacrolimus ELISA kit employs gravimetrically prepared standards using highly purified tacrolimus from Fujisawa Pharmaceutical Co. LTD., Osaka, Japan.

QUALITY CONTROL

Each laboratory should include at least two control samples in every assay to ensure the validity of each assay's results. If you are using unassayed controls and have not established a mean and standard deviation, a mean and standard deviation should then be determined for each control preferably using NCCLS guidelines. The DiaSorin QC laboratory has determined a range for the controls included with this kit. These ranges are printed on the control vials included with this kit.

In order for a laboratory to completely monitor the consistent performance of an ELISA assay, there are additional factors which should be monitored. DiaSorin suggests a regular check of the following parameters to assure consistent performance.

- Maximum absorbance of the Zero Standard ≥ 1.500 AU.
- The mean values of the Kit Controls be within the ranges published on the vial labels
- Parameter C from the 4PL curve fit equation < 5.0 .

In addition, acceptable sample results are recommended to conform to the following criteria:

- The duplicates of individual samples should agree within 15%.

RESULTS

Interpretation of Results

The standard curve for the DiaSorin PRO-Trac™ II Tacrolimus ELISA is obtained by plotting the log of the concentration on the x-axis vs the absorbance on the y-axis.

1. Measure mean absorbances for each standard control and sample.
2. Using log linear graph paper, plot the log of the concentration on the x-axis vs the absorbance on the y-axis.
3. Draw the best fit curve through the points.
4. Interpolate tacrolimus concentrations of the samples from the standard curve. Sample absorbance values which are not between two standards must be reported at < 0.3 ng/mL or > 30 ng/mL. Extrapolation beyond the standard curve is not valid for this curve fit.
5. In practice, most laboratories will employ a computer-based data reduction system. DiaSorin uses the MultiCalc® data reduction program utilizing a 4-parameter logistic (4PL) curve fitting. A representative standard curve is shown below.

TABLE I
DiaSorin PRO-Trac™ II Tacrolimus Sample Data

Well	Duplicate Absorbance	Average Absorbance	Concentration (ng/mL)
NSB	0.100 0.100	0.100	
0 Standard	2.626 2.582	2.604	0.0
Standards (ng/mL)			
A (0.3) [0.36 nmol/L]	2.377 2.342	2.359	0.3
B (1.0) [1.22 nmol/L]	2.000 1.944	1.972	1.0
C (3.0) [3.64 nmol/L]	1.293 1.287	1.290	3.0
D (10.0) [12.16 nmol/L]	0.567 0.548	0.558	10.0
E (30.0) [36.50 nmol/L]	0.292 0.297	0.294	30.0
Unknown Samples			
1	1.476 1.426	1.451	2.36
2	0.373 0.361	0.367	19.28

Typical sample data and a standard curve are shown in TABLE I and FIGURE 1; this information is for reference only and should not be used for the calculation of any value.

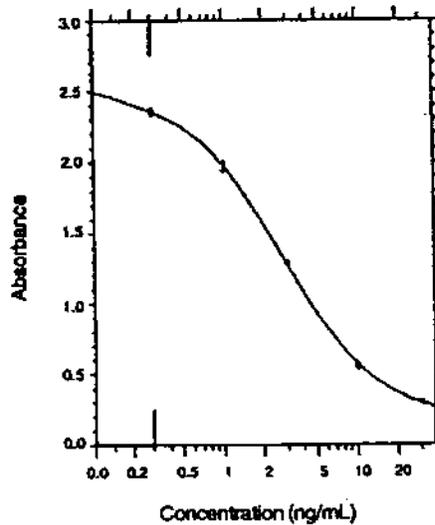


FIGURE 1
A representative standard curve obtained with the PRO-Trac™ II Tacrolimus ELISA. This information is for reference only and should not be used for the calculation of any value.

Limitations of the Procedure

- Individual tacrolimus values cannot be used as the sole indicator for making changes in the treatment regimen.
- If a sample reads greater than the highest standard, dilute the sample with Zero Standard, mix thoroughly and re-assay as a new sample. Multiply the final assay result by the appropriate correction factor. Dilutions greater than 1:4 should not be necessary.
- The PRO-Trac™ II Tacrolimus ELISA is not designed to test body fluids other than human whole blood.
- Tacrolimus values obtained by other methods may not be directly comparable.
- The immunoassay is non-specific to certain tacrolimus metabolites and cross-reactivity with certain tacrolimus metabolites has been demonstrated for the PRO-Trac™ II Tacrolimus ELISA (Table 9). When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate. In such cases confirmation of the immunoassay result by HPLC/MS/MS analysis may be desirable.
- **WARNING:** Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which utilize mouse monoclonal antibodies. These specimens should not be assayed with the PRO-Trac™ II Tacrolimus assay.

EXPECTED VALUES

No therapeutic range is established in the current clinical study for effective tacrolimus concentration in whole blood. Absorption and clearance of tacrolimus can vary greatly among patients. Clinical response to tacrolimus treatment does not correlate well with the administered dose. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic and toxic effects of tacrolimus, coadministration of other immunosuppressants, time post transplant, and a number of other factors will result in different requirements for optimal blood levels of tacrolimus. Individual tacrolimus values can not be used as the sole indicator for making changes in the treatment regimen.

A prospective clinical evaluation using the PRO-Trac™ II Tacrolimus ELISA was conducted at six clinical sites within the United States. These sites evaluated trough levels of tacrolimus in 111 liver transplant patients. Trough levels were monitored for up to 12 weeks post-transplant. Mean trough levels across all sites were 10.4 ± 6.1 ng/mL for Week 1, trending downward to 8.1 ± 3.8 ng/mL at Week 12. Over the 12 week post-transplant evaluation period, incidence rates for monitored primary clinical endpoints were: Acute graft rejection (confirmed by histology), 36 subjects (32.4%), nephrotoxicity defined as increased serum creatinine levels at least twofold greater than baseline, 38 subjects (34.2%), and any toxicity requiring a reduction in Prograf® dosage, 10 subjects (9.0%). The data demonstrated that there was not a clear relationship between tacrolimus dosing, whole blood concentrations of tacrolimus and adverse events of rejection and nephrotoxicity. The incidence of adverse events declined from 36.9% the first week to 0.9% by Week 12.

The Lake Louise Consensus Report indicated that though the therapeutic range for tacrolimus has not been established, targeted 12 hour trough concentrations were suggested to be 5 - 20 ng/mL. The incidence of adverse events increases with higher whole blood tacrolimus concentrations.*

PERFORMANCE DATA

NOTE: The performance data was generated using the following instrumentation: Denley Wellprep™ 4 plate washer, DiaSorin XL 5000 plate reader, Bio-Tek Instrument ELP-40 Microplate Strip Washer, Bio-Tek Instrument ELx 800 Microplate Reader.

Sensitivity

Analytical Sensitivity

The sensitivity of the PRO-Trac™ II Tacrolimus ELISA was determined from multiple assays using multiple lots of materials throughout the shelf-life of the kit. The sensitivity was calculated as the concentration corresponding to the absorbance at 2 standard deviations from the Zero Standard absorbance. The calculated analytical sensitivity is 0.27 ng/mL.

Functional Sensitivity

Functional sensitivity is defined as the concentration at which the precision, expressed as %CV, exceeds 20%, as determined from a plot of the non-linear regression of the mean concentration vs %CV. Functional sensitivity was determined by diluting patient samples with Zero Standard into the range of 0.5 to 2.0 ng/mL. These samples were then assayed in replicates of 8 over 3 assays to determine an inter-assay %CV. The functional sensitivity for this assay is estimated at 1.0 ng/mL.

As confirmation of the non-linear regression, inter-assay precision was determined for low concentration samples (1 – 5 ng/mL). Spiked whole blood samples were prepared at the target concentrations of 1, 3, and 5 ng/mL, aliquoted and analyzed with results presented in Table 2.

TABLE II Inter-Assay Precision Using Low Tacrolimus Concentration Levels

Lot #	N	1 ng/mL		3 ng/mL		5 ng/mL		Mean	SD	%CV
		Mean	SD	Mean	SD	Mean	SD			
1	50	1.0	0.1	11.3	2.9	0.2	5.7	5.2	0.4	8.0
2	50	0.9	0.1	12.4	2.7	0.2	7.1	4.8	0.3	6.7
3	27	0.8	0.1	17.6	2.7	0.3	10.4	4.7	0.6	2.0
Total	127	0.9	0.1	15.2%	2.8	0.2	8.6%	4.9	0.5	9.3%

Precision

Using NCCLS performance guideline EP5-T2, internal precision studies using EDTA whole blood samples spiked with tacrolimus at three concentration levels, were evaluated running one assay per day over twenty operating days. Within run and total precision for three lots were estimated by analysis of variance (ANOVA). Within the range of concentrations from 5 to 20 ng/mL, the within run imprecision is < 10% and the total imprecision is < 15%.

TABLE III Representative PRO-Trac™ II ELISA Precision Performance Evaluation Internal Study

Sample/Analysis	N	Mean	SD	%CV
Level 1				
Within-Run	80	3.8	0.3	7.5%
Total			0.3	8.8%
Level 2				
Within-Run	80	8.0	0.5	6.5%
Total			0.6	7.2%
Level 3				
Within-Run	80	16.3	1.4	8.7%
Total			1.6	9.8%

Data for one representative lot

Using the NCCLS guideline EP5-T2 as reference, ten clinical samples spanning a range of clinically relevant concentrations were evaluated by three clinical sites. Table 4 shows data from all sites.

Table IV Inter-Assay Precision at Clinical Sites

Sample #	Site 1				Site 2				Site 3			
	Mean	SD	%CV	N	Mean	SD	%CV	N	Mean	SD	%CV	N
1	13.4	1.4	10.3	34	12.4	1.3	10.5	50	11.0	1.4	12.4	40
2	17.3	1.8	10.6	33	15.6	1.6	9.9	54	14.9	1.5	10.3	39
3	9.7	1.0	10.3	33	8.6	1.0	11.8	52	7.5	1.0	13.3	40
4	4.0	0.6	14.7	36	3.8	0.5	14.2	55	2.9	0.5	16.6	38
5	5.2	0.9	17.1	36	5.2	0.7	12.5	56	4.2	0.5	11.1	34
6	8.3	1.2	14.0	36	7.9	0.9	11.1	54	6.5	0.9	13.6	38
7	12.9	1.6	12.2	34	12.6	1.4	10.7	55	11.5	1.5	13.1	39
8	10.4	1.1	11.1	36	9.9	1.0	9.6	52	8.4	0.9	10.2	40
9	10.8	1.4	13.0	35	10.5	1.1	10.1	52	9.3	1.0	10.7	40
10	17.7	1.9	10.8	34	17.2	1.8	10.4	52	15.4	1.6	10.1	40
Mean %CV			12.4 ± 2.3				11.1 ± 1.4				12.1 ± 2.1	

During the clinical trials, kit controls were assayed with each analysis, per the package insert instructions. Table 5 summarizes the precision of the controls over the course of the trials.

Table V Kit Control Precision at Clinical Sites

		Site A	Site B	Site C
Kit Control 1	n	43	67	21
	Mean	2.2	2.0	2.1
	SD	0.31	0.13	0.27
	Range	1.5	0.7	1.0
	%CV	14.5	6.8	12.9
Kit Control 2	n	43	67	21
	Mean	14.9	14.0	14.8
	SD	1.87	1.03	1.25
	Range	8.4	5.2	4.6
	%CV	12.5	7.4	8.4

Dilution Linearity

Dilution Linearity was assessed by diluting 3 high concentration spiked whole blood samples and 5 clinical samples with Zero Standard and assaying. The results were plotted as a linear regression of the Expected Value vs the Observed Value. Dilution linearity demonstrates a functional assay range from 1.0 to 30.0 ng/mL.

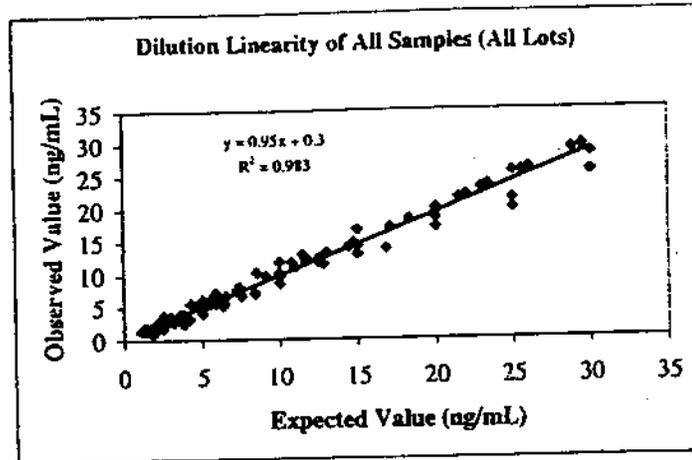


FIGURE II
 Dilution Linearity of Whole Blood Samples. Three spiked whole blood samples and five clinical samples were diluted with Zero Standard and assayed. The resulting regression line is $y = 0.95x + 0.3$; $r = 0.99$.

Recovery

Recovery was assessed by an addition of standard method. Ten clinical samples with HPLC/MS/MS values were selected and diluted 1:2 with Zero, C, D, or E Standard and assayed in duplicate. Neat clinical samples (in duplicate) were included in the same assay. The overall mean % recovery by this method was $114\% \pm 12\%$ (range 86% - 163%).

TABLE VI Percent Recovery By Standard Addition

Sample	Neat Values (ng/mL)		Measured Concentrations Diluted 1:2 With Standard (HPLC/MS/MS Value ng/mL)				Mean % Rec.
	HPLC*	Assay	0 Std (0)	C Std (3.2)	D Std (9.8)	E Std (32.4)	
1	1.4	1.9	1.1 (157%)	2.5 (109%)	6.6 (118%)	16.9 (100%)	121%
2	3.4	3.8	2.0 (118%)	3.4 (103%)	7.3 (111%)	16.7 (93%)	106%
3	5.1	5.3	2.2 (86%)	4.0 (96%)	9.3 (125%)	21.5 (115%)	106%
4	5.9	6.5	4.2 (142%)	4.7 (103%)	9.5 (121%)	20.9 (109%)	119%
5	10.2	13.2	7.7 (151%)	7.9 (118%)	11.7 (117%)	21.1 (99%)	121%
6	10.2	10.4	5.3 (104%)	6.4 (96%)	10.5 (105%)	19.5 (92%)	99%
7	13.8	13.3	7.2 (104%)	8.1 (95%)	12.2 (103%)	21.4 (93%)	99%
8	16.2	21.8	13.2 (163%)	13.3 (137%)	16.9 (130%)	27.7 (114%)	136%
9	23.6	27.1	16.0 (136%)	14.7 (110%)	17.5 (105%)	27.9 (100%)	113%
10	24.5	31.2	16.9 (138%)	16.2 (117%)	20.2 (118%)	29.4 (103%)	119%
Mean % Recovery			130%	108%	115%	102%	114%

* HPLC/MS/MS values for clinical samples and standard values were utilized to determine the % recovery

In a second recovery design known amounts of tacrolimus were added to an EDTA whole blood pool at low concentration (5 ng/mL), an intermediate concentration (10 ng/mL), and a high concentration (20 ng/mL). The overall mean % recovery by this method was 81% ± 4%.

TABLE VII Percent Recovery of EDTA Whole Blood Spiked with Tacrolimus

Spiked/Target Concentration	Observed Mean	% Recovery	
	Concentration (N=40)	Mean	Range
5 ng/mL	3.9 ± 0.2 ng/mL	78%	70% - 90%
10 ng/mL	8.2 ± 0.4 ng/mL	82%	74% - 94%
20 ng/mL	16.9 ± 0.8 ng/mL	84%	75% - 93%

Interfering Compounds and Cross-reactivity

Interference was evaluated by spiking test compounds at concentration approximately 2.5 times the expected therapeutic level into whole blood containing tacrolimus. Evaluation was consistent with NCCLS guidelines (EP7-P). Test spiked samples and the respective controls were assayed in replicates of five each. Compounds were determined to be non-interfering if the mean test concentration was within 15% of the mean control concentration. None of the drugs listed below exhibited interference in this assay at the concentrations tested.

TABLE VIII Compounds Tested for Potential Interference

Drug Tested	Concentration Tested	Drug Tested	Concentration Tested
Acetaminophen	40 µg/mL	Ganciclovir*	1000 µg/mL
Acyclovir*	1000 µg/mL	Itraconazole	10 µg/mL
Amikacin	60 µg/mL	Ketoconazole	10 µg/mL
Amphotericin B	20 µg/mL	Lidocaine	10 µg/mL
Azathioprine	1 µg/mL	Mycophenolic acid	600 ng/mL
Azithromycin*	5 µg/mL	Methylprednisolone	100 µg/mL
Carbamazepine	20 µg/mL	Nifedipine	60 ng/mL
Cefazolin	150 µg/mL	Penicillin	100 µg/mL
Ceftriaxone	500 µg/mL	Phenobarbital	80 µg/mL
Cimetidine	10 µg/mL	Phenytoin	40 µg/mL
Clarithromycin	5 µg/mL	Prednisone	10 µg/mL
Cyclosporine*	1000 ng/mL	Ranitidine	1 µg/mL
Digoxin	5 ng/mL	Rapamycin	5 ng/mL
Erythromycin	5 µg/mL	Tobramycin	20 µg/mL
Famotidine	10 µg/mL	Trimethoprim	5 µg/mL
Fluconazole	15 µg/mL	Sulfamethoxazole	150 µg/mL
Furosemide	100 µg/mL	Valproic acid	200 µg/mL
Gentamicin	20 µg/mL	Verapamil	1 µg/mL

* Tested at 10 times the maximal therapeutic level

Metabolite cross-reactivity was determined by adding 5 ng/mL of metabolite to whole blood samples containing 5 ng/mL of parent drug and assaying. Cross-reactivity was calculated according to NCCLS guidelines (EP7-P). The metabolite cross-reactivity is consistent with previously published reports for this antibody.¹⁴

TABLE IX Immunological Cross-Reactivity of Tacrolimus Metabolites

Metabolite	Modification	Cross-Reactivity PRO-Trac™ II
M-I	(13-O-demethyl-)	0%
M-II	(31-O-demethyl-)	85%
M-III	(15-O-demethyl-)	38%
M-IV	(12-O-hydroxyl-)	0%
M-V	(15,31-O-didemethyl-)	44%
M-VI	(13,31-O-didemethyl-)	0%
M-VII	(13,15-O-didemethyl-)	0%
M-VIII	(31-O-demethyl, rearrangement)	ND

ND = not determined

Potential interfering endogenous substances were added at the following final concentrations: Bilirubin, 0.4 mg/mL; Protein (added as Human Serum Albumin), 25 mg/mL; Triglycerides, 8.0 mg/mL and Uric Acid, 0.4 mg/mL. Each test sample was assayed in duplicate in 3 assays over 2 days. Elevated protein or bilirubin may affect sample results.

TABLE X Interference by Endogenous Substances

Potential Interferent	Concentration Tested	Mean Control Value (\pm SD) ng/mL	Mean Test Value (\pm SD) ng/mL	Difference (ng/mL)	% Error
Triglycerides	8.0 mg/mL	3.0 \pm 0.4 ng/mL	2.6 \pm 0.3 ng/mL	0.4	13%
		9.9 \pm 0.3 ng/mL	9.2 \pm 0.5 ng/mL	0.7	7%
		18.1 \pm 0.8 ng/mL	16.2 \pm 0.7 ng/mL	1.9	10%
Uric Acid	0.4 mg/mL	3.0 \pm 0.1 ng/mL	2.6 \pm 0.2 ng/mL	0.4	13%
		9.5 \pm 0.0 ng/mL	9.8 \pm 0.7 ng/mL	0.3	3%
		19.0 \pm 0.5 ng/mL	18.0 \pm 1.2 ng/mL	1.0	5%
Bilirubin	0.4 mg/mL	3.0 \pm 0.1 ng/mL	3.6 \pm 0.3 ng/mL	0.6	20%
		9.5 \pm 0.0 ng/mL	11.5 \pm 0.4 ng/mL	2.0	21%
		19.0 \pm 0.5 ng/mL	21.1 \pm 0.5 ng/mL	2.1	11%
Protein (as human serum albumin)	25.0 mg/mL	3.0 \pm 0.1 ng/mL	2.3 \pm 0.2 ng/mL	0.7	23%
		9.5 \pm 0.0 ng/mL	8.2 \pm 0.2 ng/mL	1.3	14%
		19.0 \pm 0.5 ng/mL	17.6 \pm 1.3 ng/mL	1.4	7%

Method Correlations

Ninety-five EDTA whole blood samples from thirty-five patients taking tacrolimus were analyzed using the PRO-Trac™ II Tacrolimus ELISA, a recently approved microparticle EIA (MEIA), and HPLC/MS/MS. Comparisons between methods are shown below. The data was analyzed by scatter plot and Bland/Altman analysis.

The PRO-Trac™ II Tacrolimus ELISA was compared to HPLC/MS/MS by scatter plot (Figure 3) including the line of identity and Bland/Altman analysis (Figure 4).

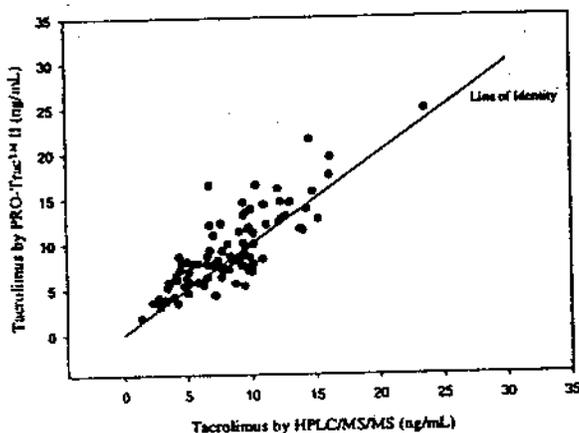


FIGURE III
Method Comparison: HPLC/MS/MS vs PRO-Trac™ II ELISA. Clinical samples (N = 95) were assayed by both HPLC/MS/MS and PRO-Trac™ II ELISA. Results are presented in comparison to the line of identity.

Method Comparison by Bland/Altman Analysis
HPLC/MS/MS vs PRO-Trac™ II

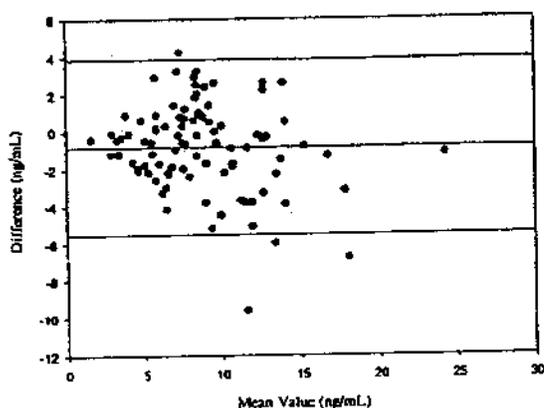


FIGURE IV
Method comparison by Bland/Altman analysis: HPLC/MS/MS vs PRO-Trac™ II ELISA. Clinical samples (N = 95) were analyzed by both methods. The mean difference (\pm SD) between methods was -0.8 ± 2.3 ng/mL.

The PRO-Trac™ II Tacrolimus ELISA was compared to MEIA by scatter plot (Figure 5) including the line of identity and Bland/Altman analysis (Figure 6) on the same 95 samples from 35 patients.

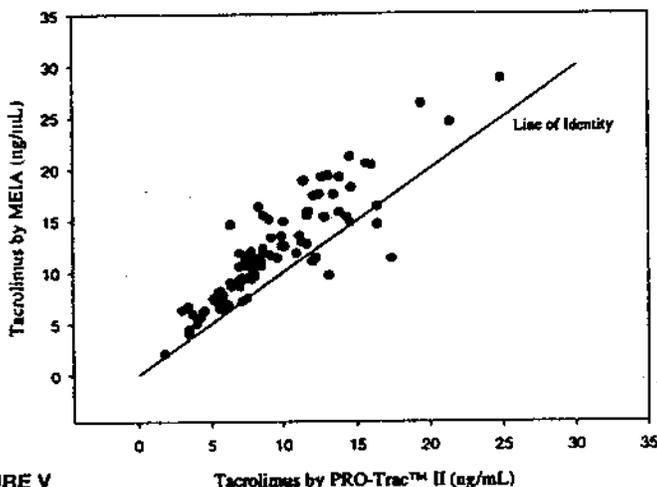


FIGURE V
 Method comparison: PRO-Trac™ II ELISA vs MEIA. Clinical samples (N = 95) were assayed by both PRO-Trac™ II ELISA and MEIA. Results are presented in comparison to the line of identity.

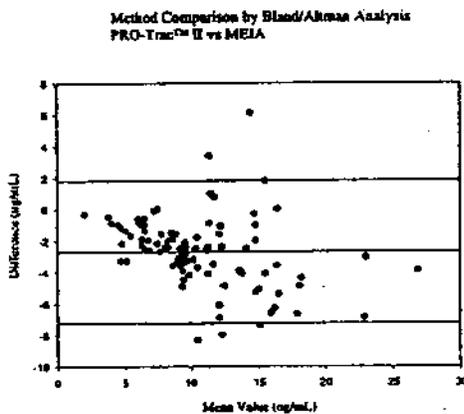


FIGURE VI
 Method comparison by Bland/Altman analysis: PRO-Trac™ II ELISA vs MEIA. Clinical samples (N = 95) were analyzed by both methods. The mean difference (\pm SD) between methods was -2.7 ± 2.3 ng/mL.

SEE LAST PAGE FOR REFERENCES

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Plate Map/Plaque de Carte/Plattenanordnung

	1	2	3	4	5	6	7	8	9	10	11	12
A	NSB	C Std.	KC 2	O	O	O	O	O	O	O	O	O
B	NSB	C Std.	KC 2	O	O	O	O	O	O	O	O	O
C	O Std.	D Std.	O	O	O	O	O	O	O	O	O	O
D	O Std.	D Std.	O	O	O	O	O	O	O	O	O	O
E	A Std.	E Std.	O	O	O	O	O	O	O	O	O	O
F	A Std.	E Std.	O	O	O	O	O	O	O	O	O	O
G	B Std.	KC 1	O	O	O	O	O	O	O	O	O	O
H	B Std.	KC 1	O	O	O	O	O	O	O	O	O	O

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