

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

**A. 510(k) Number:**

k122420

**B. Purpose for Submission:**

New assay

**C. Measurand:**

25-Hydroxy-vitamin D

**D. Type of Test:**

Quantitative, competitive enzyme immunoassay

**E. Applicant:**

Diazyme Laboratories

**F. Proprietary and Established Names:**

25-OH Vitamin D EIA Kit

25-OH Vitamin D EIA Control Kit

**G. Regulatory Information:**

<b>Product</b>	<b>Classification</b>	<b>Regulation Section</b>	<b>Panel</b>
MRG	Class II	21 CFR 862.1825 Vitamin D Test System	Clinical Chemistry (75)
JJX	Class I, reserved	21 CFR 862.1660 Quality Control Material	Clinical Chemistry (75)

**H. Intended Use:**

1. Intended use(s):

See indication(s) for use below.

2. Indication(s) for use:

The Diazyme 25-OH Vitamin D EIA is designed for the quantification of total 25-OH Vitamin D in human serum and plasma. The assay results are to be used in parallel with other clinical data to assess the Vitamin D status of a patient. For *in vitro* diagnostic use only.

The 25-OH Vitamin D EIA Control Kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D EIA Kit only. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

96-well microplate reader that reads at 450 nm.

**I. Device Description:**

25-OH Vitamin D EIA Kit

The device is a ready-to-use, six-reagent kit. Including an Extraction Solution (EX), Wash buffer (Wash 20X), and STOP solution (HCl). Reagent 1 is PBS containing sheep monoclonal antibody to 25-OH Vitamin D, stabilizers and preservatives. Reagent 2 contains PBS containing anti-sheep IgG linked to HRP, stabilizers and preservatives. Reagent 3 contains a proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide. A 96-well microplate strip coated with a 25 OH-D derivative is included in the kit. The kit contains six levels of ready-to-use calibrator material from human serum supplied in aliquots of 1.0 mL (6x1 mL vials are provided per kit).

25-OH Vitamin D EIA Control Kit

Two levels of ready-to-use control material (26.0 and 52.3 ng/mL) are provided in the kit. Controls are manufactured from human serum and contains sodium azide (<0.1%), supplied in aliquots of 1.0 mL (2x1 mL vials are provided with the kit).

Calibrators and controls contain human source material were tested by FDA-approved methods and found negative for the Human Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV).

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

IDS 25-OH Vitamin D EIA

2. Predicate 510(k) number(s):

k021163

3. Comparison with predicate:

Similarities and differences:

	<b>IDS 25-OH Vitamin D EIA Predicate (k021163)</b>	<b>Diazyme 25-OH Vitamin D EIA</b>
Intended Use	For the quantitative determination of 25 hydroxyvitamin D (25-OH D) and other hydroxylated metabolites in human serum or plasma.	Same
Type of test	Quantitative	Same
Method	96-well microplate	Same
Specimen type	Human serum or plasma.	Same
Measuring range	2.4 – 144 ng/mL	8.3 – 143.6 ng/mL
Calibrator	Serum based, liquid stable ready to use, 6 levels	Same
Control	Serum based, liquid stable ready to use, 2 levels	Same

**K. Standard/Guidance Document Referenced (if applicable):**

CLSI EP5-A2 Evaluation of Precision Performance of Clinical Devices

CLSI EP6-A Evaluation of the Linearity of Quantitative Analytical Methods

CLSI EP7-A2 Inference Testing in Clinical Chemistry

CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline

**L. Test Principle:**

Assay is based on the competition (for a 25-OH Vitamin D antibody) between a 25-OH Vitamin D conjugate coated on a microplate and the 25-OH Vitamin D content of a serum sample. Following kit instructions, Vitamin D is extracted using reagent EX and deep-well pre-dilution strips. The extracted Vitamin D samples are transferred to the coated microplate. Reagent R1 containing the 25-OH Vitamin d antibody is added to the microplate. After an incubation and a wash step, R2 containing the HRP-labeled secondary antibody is added to the microplate. Next is a second incubation followed by the addition of R3 containing the TMB substrate. After a final incubation the reaction is stopped using the STOP solution and the colorimetric signal is read. The 25-OH Vitamin D concentration of a patient sample is inversely proportional to the measured absorbance at 450 nm. Assay time is 2 hours.

**M. Performance Characteristics (if/when applicable):**

Performance was tested using a Dynex automated microplate reader.

1. Analytical performance:

*a. Precision/Reproducibility:*

The sponsor performed precision studies in accordance with the CLSI EP5-A2 guideline. Over 20 days, 10 precision levels including 2 serum based control samples (26.0 ng/mL and 52.3 ng/mL vitamin D) and 8 serum samples from 10.5 ng/mL to 116.2 ng/mL vitamin D were measured daily. Each sample was run in replicates of 2 per run, 2 runs per day for a total of 80 results per sample. Precision results are summarized in the table below:

Specimen	n	Concentration (ng/mL)	Within-run SD (ng/mL)	Within-run CV (%)	Total SD (ng/mL)	Total CV (%)
Control #1	80	26.0	0.97	3.7	1.55	6.0
Control #2	80	52.3	1.43	2.7	3.93	7.5
Low	80	10.5	1.39	13.3	1.84	17.6

sample #1						
Low sample #2	80	12.7	1.14	9.0	2.04	16.0
Sample #1	80	23.3	1.65	7.1	1.89	8.1
Sample #2	80	40.0	3.70	9.2	3.75	9.4
Sample #3	80	52.8	1.18	2.2	4.03	7.6
Sample #4	80	69.3	1.94	2.8	6.03	8.7
Sample #5	80	84.1	4.69	5.6	6.33	7.5
Sample #6	80	101.4	4.65	4.6	5.68	5.6
Sample #7	80	116.8	4.89	4.2	5.77	4.9
Sample #8	80	116.2	2.98	2.6	6.43	5.5

*b. Linearity/assay reportable range:*

The claimed linearity range of this device is 8.3 ng/mL to 143.6 ng/mL.

The sponsor performed a linearity study in accordance with the CLSI EP6-A guideline using one lot of calibrator and one lot of reagent. Eleven levels of serum based samples (5.0, 12.03, 19.06, 33.12, 47.18, 61.24, 75.3, 89.36, 103.42, 117.48, 145.6 ng/mL) were prepared by diluting a high serum sample containing 145.6 ng/mL of 25-OH Vitamin D. Samples were measured in triplicate. The assay was linear between 6.7 ng/mL and 143.6 ng/mL. The results between the expected concentrations and the measured concentrations yielded a linear regression of  $y=1.005x + 1.1674$ .

Based on the results of the LoQ and linearity studies, the sponsor's claimed measuring range is 8.3 ng/mL to 143.6 ng/mL.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

The master calibrator batch stock was prepared in house gravimetrically in DMSO. The value assignment of the stock is made by UV absorbance spectrometry calibrated with NIST SRM 935a, based on NIST recommended molar extinction coefficients. The DMSO stock of 25-OH Vitamin D3 was used to make calibration levels by dilution into pooled human serum.

Value assignments

Normal (higher than 30 ng/mL) and Abnormal (lower than 29.9 ng/mL) 25-OH Vitamin D controls prepared in human serum were tested with released Diazyme 25-OH Vitamin D kits in 120 replicates, obtained from 5 independent runs, on 2 automatic analyzers. The obtained mean values were assigned as control target values. The controls target ranges were assigned as mean  $\pm$  25% (ng/mL).

Calibrator value assignments are based on an internal procedure. The initial value

assignment for calibrators was performed using the predicate assay. Subsequent patient samples were tested against the predicate assay and the final calibrator values were adjusted and verified.

### Stability

Stability study was based on accelerated study and real-time stability study is on-going. Stability study protocol and acceptance criteria has been provided and found to be adequate. The kit is stable until the expiration date if stored at 2-8 °C. Calibrators, controls, and the 25-D Biotin solution are stable for 8 weeks at 2-8 °C. If opened, the unused antibody coated microplate is stable for 8 weeks at 2-8 °C only if returned to the foil pouch and sealed. The 20X wash solution is stable at room temperature for 8 weeks and the 1X working wash solution is stable at room temperature for 4 weeks.

#### *d. Detection limit:*

A detection limits study was performed by the sponsor based on the CLSI EP17-A guideline. To determine the LoB, serum containing undetectable levels of 25-OH Vitamin D from a commercial source was assayed in three independent runs with 20 replicates per run (N=60). The LoB was calculated as the mean 57<sup>th</sup> and 58<sup>th</sup> highest values for the blanks. LoB is determined to be 3.0 ng/mL

To determine the LoD five low Vitamin D serum samples were measured in three independent runs, with four replicates per run (N=60). The LoD is defined as  $LoD = LoB + (1.645 * STDEV \text{ of Low samples})$ . LoD is determined to be 5.6 ng/mL.

The LoQ was measured using five samples (6.7, 12.4, 18.3, 22.3, 28.8 ng/mL). Each sample was assayed in 40 replicates obtained in five independent runs. LoQ is defined as the lowest concentration for which %CV is <20%. A curve fit was used to obtain an estimate of %CV as a function of mean. LoQ was determined to be 8.3 ng/mL.

The sponsor's claimed measuring range is 8.3 to 143.4 ng/mL.

#### *e. Analytical specificity:*

##### Interference study:

An interference study was performed by the sponsor to evaluate different potential interference substances. Two unaltered serum samples (one deficient and one normal) were used for the testing. One aliquot of the samples were spiked with 5 concentrations of potential interfering substances and an aliquot of unspiked samples were used as a control. All samples were tested in duplicates. The sponsor defined non-significant interference as within  $\pm 10\%$  difference between the spiked and unspiked samples.

The interference substances examined and their concentrations tested are listed in the following tables:

Interference Substances	Concentration tested				
	C1	C2	C3	C4	C5
Ascorbic acid (mM)	0.0	1.0	2.5	5.0	10.0
Bilirubin (mg/dL)	0.0	10	20	30	40
Bilirubin Conjugated (mg/dL)	0.0	10	20	30	40
Hemoglobin (mg/dL)	0.0	50	100	250	500

Interference Substances	Concentration tested				
	Triglycerides (mg/dL)	0.0	250	300	350
		450	500	750	1000

Following is the list of interference substances tested with the highest concentrations of interference substances that did not have a significant interference with the assay.

Conjugated bilirubin	40 mg/dL
Free bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Ascorbic acid	10 mM
Triglycerides	450 mg/dL

The sponsor states the following in their labeling: “Do not use hemolysed samples.”

Cross-reactivity study:

To test for cross-reactivity with potential cross-reactants, six identical serum aliquots (10 mL) were each spiked with one of the cross-reactant listed below. Cross-reactants stock solutions were made with 100% DMSO. A seventh serum aliquot of 10 mL was spiked with 100% DMSO (devoid of Vitamin D) and served as a negative control. All 7 spiked sera were then quantified in quadruplicates. % Cross-reactivity was calculated using the following the equation:

$$\% \text{ Cross-reactivity} = \frac{\text{spiked sample} - \text{unspiked sample}}{\text{conc. of cross reactants spiked}} \times 100$$

The following were tested and results were summarized in the table below:

Cross-reactant	Unspiked Vitamin D (ng/mL)	Spiked Vitamin D (ng/mL)	% Cross-reactivity
25-OH Vitamin D3 (tested at 63 ng/ml)	24.4	87.4	100.0%
25-OH Vitamin D2 (tested at 63 ng/ml)	24.4	85.4	96.8%
Vitamin D3 (tested at 63 ng/ml)	24.4	24.2	-0.3%
Vitamin D2 (tested at 63 ng/ml)	24.4	22.3	-3.3%
1,25-OH Vitamin D3 (tested at 63 ng/ml)	24.4	84.1	94.8%
1,25-OH Vitamin D2 (tested at 63 ng/ml)	24.4	62.0	59.7%
24R,25-OH Vitamin D3 (tested at 56 ng/mL)	9.8	23.2	23.9%
3-epi-25-OH Vitamin D3 (tested at 57 ng/ml)	21.2	69.6	84.9%
3-epi-25-OH Vitamin D2 (tested at 57 ng/ml)	21.2	59.9	67.9%

*f. Assay cut-off:*

N/A

2. Comparison studies:

*a. Method comparison with predicate device:*

A method comparison study was performed using the candidate device (Diazyme 25-OH vitamin D EIA) and the predicate device. The obtained results from the candidate device were compared to those of the predicate device. 58 serum samples were tested with seven spiked samples to cover the high end measuring range. The samples range tested was from 11.9 to 131.5 ng/mL. Deming regression yielded the following results.

Slope = 0.9341 (CI: 0.906 to 1.045), y-intercept = +1.448 (CI: -3.83 to 3.7),  $r^2 = 0.930$  (CI: 0.940 to 0.979)

*b. Matrix comparison:*

The sponsor performed a matrix comparison study using 66 matched sets of serum (ng/mL), Li-heparin (ng/mL), and K<sub>3</sub>-EDTA (ng/mL) to evaluate the effect of the anticoagulants on the Diazyme 25-OH Vitamin D EIA kit results. The reported values for each sample and for each matrix were obtained from single measurements. The total number of matched sets tested was 66. Seven spiked patient samples were included in the study to cover the hard-to-find samples range. The linear regression results yielded the following comparisons results for each matrix.

- Li-Heparin vs. Serum:  $y = 0.993x + 1.855$  (sample range tested was 10.9 to 130.2 ng/dL) and  $r^2 = 0.961$ .
- K<sub>3</sub>- EDTA vs. Serum:  $y = 1.006x + 2.901$  (sample range tested was 8.3 to 119.5 ng/dL) and  $r^2 = 0.967$ .

The sponsor demonstrated lithium heparin plasma and K3 EDTA plasma are acceptable anticoagulants to be used with their assay.

3. Clinical studies:

a. *Clinical Sensitivity:*

N/A

b. *Clinical specificity:*

N/A

c. *Other clinical supportive data (when a. and b. are not applicable):*

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

To determine a reference range for the Diazyme 25-OH Vitamin D EIA, the 25-OH Vitamin D serum concentrations of a U.S. population of 157 apparently healthy individuals (age between 21 and 80 years old), were measured with the Diazyme method. These samples were collected from three different geographical locations, with 47 samples collected from Pennsylvania (Northern U.S.), 56 samples collected from Tennessee (Central US) and 54 samples collected from Texas (Southern US). The study population consisted of 72 light skin individuals (46%) and 85 dark skin individuals (54%). There was no family history of parathyroid or calcium regulatory disease. There was no participant history of kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, calcium related disease, seizures,

chronic disease, or bariatric surgery. No participants were taking medication known to affect absorption or catabolism of Vitamin D. All serum samples were collected during the fall season (October 2010-November 2010).

The following results were obtained:

Lowest 25-OH Vitamin D concentration: 8.4 ng/mL

Highest 25-OH Vitamin D concentration: 61.3 ng/mL

Median 25-OH Vitamin D concentration: 29.1 ng/mL

Observed range (2.5th to 97.5th percentile): 12.0 to 55.0 ng/mL

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.