3. Linearity

Three different patient samples were diluted with the sample diluent to 1/2, 1/4, 1/8 and 1/16. HE4 values were calculated and results were corrected with the dilution factor.

	Original Value	Percentage of Recovery			
Serum	PM	1/2	1/4	1/8	1/16
1	208.2	108.7%	110.5%	107.6%	118.3%
2	739.8	91.6%	91.5%	97.6%	96.0%
3	677.8	95.8%	104.9%	110.0%	106.6%

4. Spiking Recovery

Patient sera were spiked with known amounts of HE4 and assayed spiked and un-spiked in duplicate in the same assay. Results were as follows:

Endogenous HE4 (PM)	HE4 Spiked (PM)	Expected Value (PM)	Measured Value (PM)	Recovery (%)
51.3	20	71.3	73.5	103
	150	201.3	188.3	93.5
	650	701.3	738.7	105.3

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Cat #: HE367T (96 Tests)

Calbiotech Inc., | 1935 Cordell Ct., El Cajon, CA 92020
Tel (619) 660-6162 | **Fax** (619) 660-6970 | **Web** www.calbiotech.com

IVD 2° 8° CE EC REP CEpartner4U, 3951DB; 13. NL. | **Tel** +31 (0)6.516.536.26

CALBIOTECH
A Life Science Company

Human Epididymis Protein 4 (HE4) ELISA

Catalog No.: HE367T (96 Tests)

INTENDED USE

The Calbiotech, Inc. HE4 ELISA kit is intended for the quantitative determination of HE4 in human serum or plasma.

PRINCIPLE OF THE TEST

The HE4 kit is a solid phase sandwich assay method based on streptavidin-biotin principle. Standards, samples, and the biotinylated anti-HE4 antibody reagent are added into wells coated with Streptavidin. HE4 in the samples binds to the biotinylated antibody. Simultaneously, the biotinylated antibody binds to the Streptavidin coated plate. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of Peroxidase (HRP) conjugated anti- HE4 antibody reagent, a sandwich complex is formed, where the HE4 being in between the two highly specific antibodies, labeled with biotin and HRP. Unbound protein and excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of HE4 in the samples. A standard curve is prepared relating color intensity to the concentration of HE4.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. HE4 Standards: 6 vials (ready to use)	0.5ml
3. Anti-HE4 Biotin Reagent: 1 bottle (ready to use)	12ml
4. Anti-HE4 HRP Enzyme Conjugate: 1 bottle (20X)	12ml
5. TMB Substrate: 1 bottle (ready to use)	12ml
6. Stop Solution: bottle (ready to use)	12ml
7. Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8oC.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

1. Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Dispense 20µl standards, controls, and samples into appropriate wells.
3. Add 100µl of Biotin Reagent into all the wells. Shake the microplate gently for 20-30 seconds to mix.
4. Incubate for 60 minutes, at room temperature (20-25°C).
5. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
6. Add 100µl of HRP Enzyme Conjugate to all the wells.
7. Incubate for 60 minutes, at room temperature (20-25°C).
8. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
9. Add 100µl of TMB substrate to all the wells.
10. Cover the microplate and incubate for 15 minutes, at room temperature.
11. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
12. Read the absorbance in each well at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

A standard curve is constructed as follows:

1. Calculate the average absorbance values for each set of standards and patient samples.
2. To construct the standard curve, plot the mean absorbance of each HE4 standards (vertical axis) against its concentration in PM (horizontal axis).
3. Draw the best-fit curve through the plotted points.
4. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of HE4.

Example of a Typical Standard Curve

	OD450nm	Conc. (ng/mL)
Std 1	0.006	0
Std 2	0.344	20
Std 3	0.981	100
Std 4	1.392	200
Std 5	1.800	400
Std 6	2.200	800

EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines.

1. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 24 times in the same run.

Serum	No. of Replicates	Mean (PM)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	24	0.062	0.081	0.224

2. Precision

Intra-Assay

Serum	No. of Replicates	Mean (PM)	Standard Deviation	Coefficient of Variation (%)
1	16	15.92	0.46	2.86
2	16	167.77	5.19	3.10
3	16	668.35	16.86	2.52

Inter-assay

Serum	No. of Replicates	Mean (PM)	Standard Deviation	Coefficient of Variation (%)
1	16	16	0.81	5.07
2	16	167.4	5.87	3.50
3	16	659.1	33.4	5.07