RayBiotech
Empowering your proteomics
Norcross, GA 30092, United States of America

Human epididymis protein 4 (HE4) ELISA Assay Kit For in vitro diagnostics Catalog #: ELISA-HE4-96

• EXPECTED USAGE

This kit is designed for the quantitative detection of human epididymus protein 4 in serum samples. This protein is often used for the diagnosis and curative effect monitoring of ovarian cancer patients.

• DIAGNOSTIC SIGNIFICANCE

Human epididymis protein 4, belongs to the whey acidic protein four-disulfide core (WFDC) protein family and has suspected trypsin inhibitor properties. HE4 can be detected in serum, and is highly expressed in ovarian cancer tissues, but not in adjacent tissues, normal tissues, or benign tumor tissue. Ovarian cancer has the third highest incidence rate out of all female genital system cancers, but the mortality rate is the highest. HE4 has been highly reported in the literature as a high sensitivity tumor marker that is relevant in the early diagnosis of ovarian cancer, especially at the initial stage of symptom presentation. Thus, the detection or monitoring of HE4 is helpful for the diagnosis of an evaluation of curative treatment for malignant ovarian tumors[1-4].

• DETECTION PRINCIPLES

Sandwich Based ELISA Method:

Sandwich based Enzyme linked immunosorbent assay (ELISA) is based on a capture and detection antibody pair that is specific for the target of interest. Initially, a specific anti-HE4 monoclonal antibody is immobilized on the surface of a 96-well plate. Next, calibrators or samples are added into the wells of the plate. Following incubation, a secondary detection antibody for additional specificity is added. Following secondary antibody incubation, an enzymatic conjugate is added that can bind the antibody bound to the plate and create a complex of solid phase antibody-antigen-antibody. Upon addition of a coloring substrate, this enzyme reacts to produce a color relative in amount of target HE4 present in the sample.

During the enzyme reaction, the color becomes blue, and changes to yellow in the presence of the stop solution. Finally, the OD of the wells at 450nm is read on a spectrophotometric plate reader. The OD450nm value is proportional to the concentration of HE4 in the sample.

• KIT COMPONENTS

The kit contains the following components. The entire kit can be used for 96 HE4 level measurements.

Components	Size
HE4 ELISA Plate	One 96-well plate
Plate Sealing Film	2 films
HE4 Enzyme Conjugate	One 10mL bottle
HE4 Calibration Vials	Six 0.5mL bottles (S1–S6)*
Wash Concentrate 20X	One 20mL bottle
Coloring Solution	One 11mL bottle
Stop Solution	One 7mL bottle
Instruction Manual	One copy

Note: The components of different lot numbers are not interchangeable

*The HE4 Calibration Vials S1-S6 contain 40pmol/L, 80pmol/L, 160pmol/L, 320pmol/L, 640pmol/L, and 1300pmol/L of HE4 recombinant antigen, respectively.

Additional Materials Required:

- Precision pipettes
- Distilled or deionized water
- 37°C or similar incubator
- 96-well microplate reader

• STORAGE AND STABILITY

Store kit at 2 – 8° C, away from moisture, and away from light. If stored properly, the kit is good for 12 months.

• SAMPLE COLLECTION AND STORAGE

Sample Requirements:

Serum samples should be used only. Samples that are hemolyzed, lipidemic, or jaundiced should be avoided.

Sample Isolation:

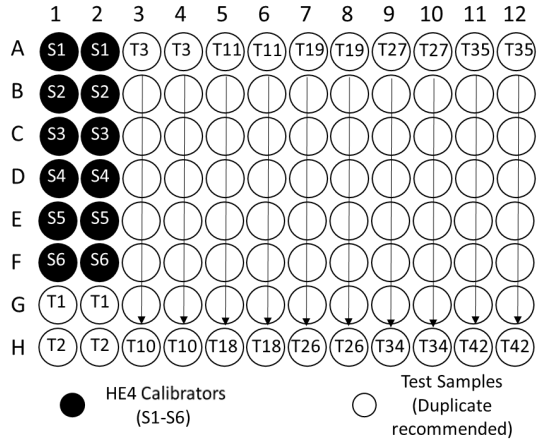
The kit is designed to test patient serum for the presence of HE4. Serum should be drawn into any appropriate tube and allowed to clot. Following clotting, the sample can be spun and the serum isolated off the clotted fraction.

Sample Preservation:

Samples should be run immediately if possible. If not, store the collected serum sample 0-4°C for 24 hours, or up to 3 months at -20°C. Avoid multiple freeze thaws.

• TESTING METHODS

1. Before testing, read the manual completely.
2. Ensure kit and sample have been stored correctly.
3. The reagents should be brought to room temperature before use. Avoid creating bubbles in reagents or sample dilutions that require mixing.
   - A standard curve should be run with each test.
   - If the sample concentration is too high, the sample should be diluted using the diluted 1x Wash Concentrate to bring it into linear range.
4. Prepare 1x Wash Concentrate by diluting the 20x Wash Concentrate 1:20 with distilled or deionized water (e.g. 20mL of 20x Wash Concentrate should be diluted with distilled water to a total volume of 400mL).
   - If crystallization occurs in the Wash Concentrate, it can be placed at 37°C to dissolve crystals before use.
   - Once reconstituted, 1x Wash Concentrate can be stored for up to 5 days at 4°C.
5. Number samples according to the corresponding microwells (example is shown below).



6. Add 20µL of the HE4 Calibrator or sample to the corresponding wells.
   - NOTE: it is recommended to run all samples and calibrators in duplicate.
7. Next, add 80µL of HE4 Enzyme Conjugate to each well and mix gently with rocking.
8. Seal the plate with the Plate Sealing Film and incubate for 60 minutes in a 37°C incubator.
   - NOTE: Plate Sealing Films are one-time-use only and cannot be reused.
9. Remove the liquid from the wells and add 100µL of 1x Wash Concentrate to each well. Repeat 5 times.

10. Next, add 100µL of the Coloring Solution to each well and mix gently with rocking at 37°C in the dark for 10 minutes.
11. Add 50µL of Stop Solution to each well and tap gently to mix.
12. Evaluate the results with a microplate reader with the wavelength set to 450nm (recommended dual-wavelength 450/630nm detection).

EVALUATION OF TEST RESULTS

Results Interpretation:

The plate should be read immediately following the addition of the Stop Solution. The concentration of HE4 in the sample is calculated from the generated calibration curve. The calibration curve should be generated by plotting the logarithm of the OD450 of each calibrator as the ordinate (Y-axis) and the logarithm of the concentration of each calibrator as the abscissa (X axis). Then, use a log-log linear fitting method determine the HE4 concentration.

Validity Evaluation:

The standard curve correlation coefficient (R^2) should not be less than 0.990 to meet the requirements of the test. The test is invalid should this value be <0.990.

Reference Range:

Normal reference values are ≤ 140pmol/L. 400 normal, human serum samples were tested with this kit and used to determine the normal reference value.

- NOTE: The normal reference range is determined by a specific method. The clinical status and individual complexity differences, regional differences, and operator differences may affect the results of the test. It is recommended that each laboratory should establish its own reference range. These data are for reference only.

Results are determined as follows:

- **Negative result:** Quantity of HE4 indicated in the sample is less than or equal to140ng/mL
- **Positive result:** Quantity of HE4 indicated in the sample is greater than 140ng/mL

Limitations of the Assay:

- This kit is only used to assist in the diagnosis of liver disease against a reference patient cohort.
- These results are subject to the sample group evaluated, and results should be combined with other liver examination to confirm the results.
- Improper storage of the kit or it's reagents can compromise the kit results.
- False Positive/Negative Result: false positives could result in the presence of reagent contamination and cross-contamination of samples.
- Accurate measurement requires that the values not exceed the range of the standard curve. Values that exceed this range should be diluted and run again

Performance characteristics:

- Specificity: the cross-reactivity with other species are as follows:

Cross original	Concentration	Detected concentration
Bilirubin	1000ng/mL	≤20pmol/L
Triglyceride	1000ng/mL	≤20pmol/L
hemoglobin	1000ng/mL	≤15pmol/L
CA125	200U/mL	≤50pmol/L
CA153	200U/mL	≤50pmol/L
AFP	1000ng/mL	≤15pmol/L
CEA	1000ng/mL	≤15pmol/L

- Minimum detection limit: 25pmol/L of HE4.
- Linear detection range: the measurable range is between 40-1300pmol/L; the correlation coefficient R of the kit should not be less than 0.99
- Accuracy: recovery should be 85% to 115%
- Repeatability: the coefficient of variation, CV ≤ 15%.
- Inter-assay Precision: the coefficient of variation, CV ≤ 15%.
- Hook Effect: at less than 2600pmol/L a high-dose Hook effect will not be observed
- Clinical testing: One thousand ninety-six clinical samples (390 positive cases and 706 negative cases) were tested. The results are as follows:

Positive coincidence rate: 379/390 = 97.2%
Negative coincidence rate: 689/709 = 97.6%
Total coincidence rate: (379+689)/1096 = 97.4%

The comparison test kit and our test kit were in good agreement.

SAFETY MEASURES

Warning statements:

- This kit is intended for use only in accordance with the instructions contained herein and is intended for use as an in vitro diagnostic test kit within the specifications and limits described in the produce description.
- This kit is limited to use in human serum samples for HE4 determination. Reliability in other body fluid samples has not yet been fully confirmed
- Concentration values obtained by other methods and the results of this assay kit are not directly comparable.
- Patients who are in close contact with a rodent or who have received a monoclonal antibody preparation in a diagnostic treatment will have tropic antibodies in their samples, which may result in abnormal results.
- All work must conform to set biological safety code regulations to prevent cross-contamination.
- Specimens and enzyme-labeled reagents should be filled with a pipette that has been calibrated for accuracy.
- Operation of this kit should be strictly in accordance with the instructions. Different lots of reagents should not be mixed.
- The kit should be used for the quantitative determination of HE4 levels in samples. Test results must be combined with patient clinical information for analysis.
- Check the product expiration and packaging integrity before use. Do not use if the product is expired or packaging is damaged.
- All tests should be done in accordance with the relevant laboratory norms and requirements promulgated by the relevant state departments strictly to prevent cross-contamination.

Waste treatment:

- Following use, this product contains animal-derived substances that may be contaminated by the sample during operation. There are no known methods to fully ensure the presence of non-infectious substances. All reagent components, samples, and various wastes should be treated as infectious agents.

REFERENCES

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5. Presl, Ceska gynekologie, 77(5), 445-449, 2012.

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