Instruction for Use

【Product Name】

Human Epididymal Protein 4 (eCLIA)

【Order Information】

REF NO.	Package Size
0320500601	50 T
0320500602	2×50 T
0320500603	100 T
0320500604	2×100 T

【Intended Use】

Immunoassay for in vitro quantitative determination of Human Epididymal Protein 4 (HE4) in human serum and plasma. Intend to clinically used for the monitoring of curative effect on ovarian cancer.

In 1991, researchers discovered Human Epididymal Protein 4 (HE4), a protein secreted by the Whey acidic protein family, in epithelial cells of the distal epididymal region. This protein, with a molecular weight of about 20-25 kD, contains a single peptide chain containing two WFDC domains[1-2]. Physiologically, it shows very low expression level in the epithelium of respiratory tract, reproductive system and ovarian tissues, but is highly expressed in ovarian cancer tissues and serum of patients[2-5]. Ovarian cancer is a deadly form of gynecologic cancer[6]. Symptoms of ovarian cancer are related to the presence of adnexal mass, but often vague and non-specific. Most ovarian cancers are detected in the advanced stage, and the 5-year survival rate of the patients with advanced ovarian cancer is less than 30.0%[7-8].

As a serum marker for pelvic tumors, HE4 has the highest sensitivity for detection of ovarian cancer, especially in Stage I disease, which is an early asymptomatic stage. The combination of CA125 with HE4 can greatly improve the detection rate of ovarian cancer, with the highest sensitivity up to 76.4% and the highest specificity up to 95.0%[9-10].

【Principles of the examination method】

Sandwich principle. Total duration of assay: 9 minutes.

15 µL of sample, biotinylated HE4 monoclonal antibody and ruthenium (Ru) complex-labeled HE4 monoclonal antibody form antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the HE4 concentration in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50 T)	Volume (50 T)	Volume (100 T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles,0.45 mg/mL; 100 mM PBS; ProClin™300	2×1.8 mL	1×1.8mL	1×3.5mL	2×3.5mL
(RB)	Biotinylated HE4 antibody, 1.25 mg/L; 100 mM PBS; ProClin™300	2×3.5 mL	1×3.5mL	1×7.0mL	2×7.0mL
(RA)	Ru complex-labeled HE4 antibody, 2.0 mg/L; 100 mM PBS; ProClin™300	2×3.5 mL	1×3.5mL	1×7.0mL	2×7.0mL
Calibrators	HE4 antigen; 100 mM PBS; ProClin™300;	High:1×1.0 mL Low:1×1.0 mL			
Control Materials (Optional)	HE4 antigen; 100 mM PBS; ProClin™300;	High:1×1.0 mL Low:1×1.0 mL			

Traceability: This method can be traced back to Roche Elecsys HE4.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzers eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002,

【Storage and Shelf Life】

Store at 2ºC~8ºC.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
on the analyzers at 2ºC~15ºC	8 weeks

The Calibrators and Control Materials are stable up to the stated expiration date.

Stability of the Calibrators and Control Materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-K2, EDTA-K3, heparin lithium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 5 hours at 18~28ºC, 4 days at 2~8ºC, and 3 months at -25ºC~-15ºC. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after two freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. HE4 testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30ºC; relative humidity: ≤ 80.0%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended as follows :

(1) When using a new lot of reagent;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) If the quality control result exceeds the defined limit;
(4) When changing the buffers of different lot numbers.

Quality Control procedure

For quality control, use HE4 control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used.

Calculation

The system software can automatically calculate the analyte concentration, with the result in pmol/L.

Specimen dilution

HE4 concentration in the sample higher than the upper limit of detection can be diluted manually with sample diluent.

The recommended dilution ratio is 1:19. (automatic dilution by instrument or manual dilution). If manual dilution, the result should be multiplied by the dilution ratio. If instrument automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Interval】

By analyzing the serum samples of 621 healthy females in hospitals, it is calculated that the reference interval is as follows:

Age	Sample size	95th percentile (pmol/L)
< 40	127	60.58
40~49	123	76.27

50~59	124	74.33
60~69	126	82.93
≥ 70	121	104.3

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 5~1500 pmol/L. If the HE4 concentration in the sample is lower than the lower limit of detection, the result is reported as < 5 pmol/L; if the HE4 concentration in the sample is higher than the upper limit of detection, the result is reported as > 1500 pmol/L (20x diluted sample is reported as > 30000 pmol/L).

When jaundice (bilirubin) ≤ 66 mg/dL, hemolysis (hemoglobin) ≤ 800 mg/dL, lipid (triglyceride) ≤ 2000 mg/dL, biotin ≤ 15 ng/mL, total protein ≤ 10 g/dL, and HAMA ≤ 100 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample ≤ 1500 IU/mL, the relative recovery of the test results is between 90% and 110%.

When the sample contains carboplatin (600 μg/mL), cisplatin (180 μg/mL), cyclophosphamide (500 μg/mL), dexamethasone (20 μg/mL), doxorubicin hydrochloride (120 μg/mL), methotrexate (150 μg/mL), paclitaxel (265 μg/mL), 5-fluorouracil (900 μg/mL), erlotinib (150 μg/mL), the interference deviation of the measured results is within ±10%.

Samples of other tumor markers containing 1000 U/mL CA125, 100 U/mL CA15-3, 1000 U/mL CA19-9, 1000 ng/mL CEA are tested, and the HE4 measured results are not greater than 15 pmol/L.

When the HE4 concentration reaches 10000 pmol/L, there is no high-dose hook effect; when the HE4 concentration reaches 40000 pmol/L, the test results are not be affected by the hook effect.

【Analytical Performance】

Limit of Blank = 5 pmol/L.

Limit of Detection = 15 pmol/L.

Accuracy

The recovery sample added with an appropriate concentration of HE4 is tested, and the recovery is between 90% and 110%.

The accuracy control subject to standardized traceability is tested, and the relative deviation of its test results is within ±10%.

Linearity

Within the range of 15 pmol/L~1500 pmol/L, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 5%.

Between-lot precision

The coefficient of variation (CV) ≤ 10%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: coefficient of variation (CV) ≤ 5%.

Between-bottle homogeneity: coefficient of variation (CV) ≤ 10%.

Accuracy of calibrators

The supporting calibrators of the kit are tested, the relative deviation of their test results is within ±10%.

Measured values of control materials

The measured results of supporting assigned quality control of the kit should be within the quality control range.

Method comparison

A comparison of the Lifotronic Human Epididymal Protein 4 (eCLIA) assay (y) with the Roche Elecsys HE4 Method (x) using clinical samples gave the following correlations:

Number of plasma samples measured: 130

Linear regression:

Y=1.0421X-3.9680

r=0.9957

The sample concentrations were between27.98 to 1496 pmol/L.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

Exercise the normal precautions required for handling all laboratory reagents.

All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.

Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Batch code		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limit		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number	P/N	Material code
	Warning		Date of manufacture
	Unique device identifier		

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