EZElisa™ Human hCG-beta/β-hCG ELISA Kit

Cat #: D-AEK6901

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Detection range: 8 IU/L-240 IU/L

Sensitivity: 2.0 IU/L

Specificity: EZElisa™ Human β-hCG ELISA Kit has high sensitivity and excellent specificity for detection of Human β-hCG. No significant cross-reactivity or interference between Human β-hCG and analogues was observed

Applicable samples: Urine, Serum, Plasm

Assay Principle

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced by trophoblastic cells of the placenta beginning 10 to 12 days after conception. Maintenance of the fetus in the first trimester of pregnancy requires the production of hCG, which binds to the corpus luteum of the ovary which is stimulated to produce progesterone which in turn maintains the secretory endometrium. hCG is present only in trace amounts in non-pregnant urine and sera. It rises sharply during pregnancy. hCG is composed of two non-identical, non-covalently linked polypeptide chains designated as the α and β subunits. The α subunit of hCG is nearly identical to that of thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and luteinizing hormone (LH). EZElisa™ Human β-hCG ELISA Kit employs a two-site sandwich ELISA to quantitate Human β-hCG in samples. An antibody specific for Human β-hCG has been pre-coated onto a microplate. Standards and samples are added to the appropriate microtiter plate wells with HRP conjugated antibody specific for Human β-hCG and any Human β-hCG present is bound by the immobilized antibody. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human β-hCG in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size（48T）	Size（96T）	Storage conditions
Human β-hCG Standard	0.25 mL×6	0.5 mL×6	4°C
HRP Conjugated Human β-hCG Detect Antibody	3 mL	6 mL	4°C
HRP Substrate A	3.5 mL	7 mL	4°C,protected from light
HRP Substrate B	3.5 mL	7 mL	4°C,protected from light
Stop Solution	3.5 mL	7 mL	4°C
Wash Buffer (20×)	7.5 mL	15 mL	4°C
Urine Sample Diluent (10×)	7.5 mL	15 mL	4°C
Human β-hCG Microplate	48 wells	96 wells	4°C
Plate Covers	1	2	RT

Note: Std1: 0 IU/L; Std2: 8 IU/L; Std3: 16 IU/L; Std4: 40 IU/L; Std5: 100 IU/L; Std6: 240 IU/L.

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 450 nm

·Multi channel pipette or automated microplate washer

·Incubator, refrigerated centrifuge

·Precision pipettes, disposable pipette tips

·Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

1×Wash Buffer: Wash buffer(20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4°C.

1×Urine Sample Diluent: Urine Sample diluent (10×) dilute with Normal saline 1:10 to obtain the 1×Urine Sample diluent. Store at 4°C.

Sample Dilution: If your samples need to be diluted, Normal saline is used for dilution of serum/plasma samples, and 1×Urine Sample diluent is used for dilution of Urine.

Sample Preparation

1.Urine: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

2.Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

3.Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20°C to avoid loss of bioactive Human β-hCG. If samples are to be used within 24 hours, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

2. Add 50 µL of Human β-hCG Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.

3. Add 50 µL of HRP conjugated Human β-hCG detect antibody to each well (not to Blank well). Mix well, cover with the plate cover provided and then incubate for 1 h at 37°C.

4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a Multi channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

5. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

6. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as HRP substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1.Average the duplicate readings for each standard and sample and subtract the average zero standard (Std1) optical density (O.D.).

2.Drawing of standard curve: With the standard solution concentration as the x-axis and the mean optical density (O.D.) for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

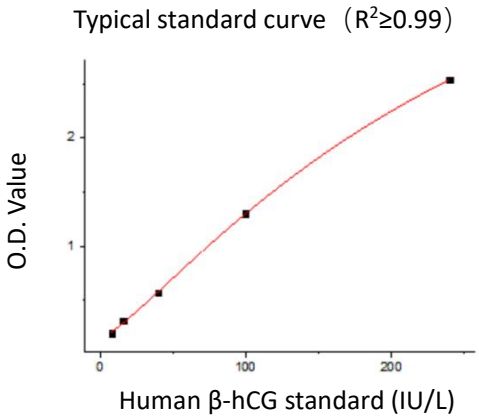

Fig.1. Standard Curve of Human β-hCG in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay

Precautions

1.Do not mix or substitute reagents with those from other lots or sources.

2.To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.

4. Stop Solution has certain Corrosive. Please take protective measures when operating.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.