antibody and ELISA experts

PicoKine™ ELISA

Catalog number: EK7062

For the quantitation of **β-hCG** concentrations in Serum and Urine.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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β-human Chorionic Gonadotropin(β-hCG) ELISA Kit

Catalog Number: EK7062

Introduction

The Bosterbio β-hCG Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure β-hCG with a 96-well strip plate that is pre-coated with antibody specific for β-hCG. The detection antibody is a HRP conjugated antibody specific for β-hCG. The capture antibody is monoclonal antibody from mouse, the detection antibody is monoclonal antibody from mouse. The kit is analytically validated with ready to use reagents. To measure β-hCG, add standards and samples to the wells, then add the HRP conjugated detection antibody. Wash away the unbounded protein and HRP conjugated detection antibody. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. Upon addition of the substrate, the density of the yellow product is linearly propotional to β-hCG in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of β-hCG in the sample. For more information on assay principle, protocols, and troubleshooting tips, see Boster's ELISA Resource Center at https://www.bosterbio.com/elisatechnical-resource-center.

Overview

Product Name	β-human Chorionic Gonadotropin(β-hCG) ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of β-hCG. 96wells/kit, with removable strips.
Sensitivity	2.0 IU/L *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	8-240 IU/L
Storage Instructions	Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Specificity	Natural and recombinant β-hCG
Cross Reactivity	There is no detectable cross-reactivity.

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Kit Components/Materials Provided

Description	Quantity	Volume	Buffers
Anti-hCG-β Pre-coated 96-well strip microplate	1	8 strips of 12 wells	Anti-hCG-β monoclonal antibody, Polystyrene micro-well plate
hCG-β Standards(S0~S5)	6	0.5ml	hCG-β (0, 8, 16, 40, 100, 240 IU/L), 0.02M PBS, 20% new-born calf serum, 0.1%Proclin-300
HRP Conjugated anti-hCG-β antibody	1	6ml	HRP Conjugated anti-hCG-β antibody, 0.02M PBS, 20% new-born calf serum, 0.01% azophloxine, 0.1%Proclin-300
Controls	2	0.5ml	hCG-β, 100% natural protein, 0.1%Proclin-300
10 X Urine Sample Dilute Buffer Concentrate	1	15ml	0.2M PBS, 20% new-born calf serum, 0.1% Proclin-300
20X Wash Buffer Concentrate	1	15ml	0.2M PBS containing 0.5% tween 20
Color Developing Reagent A	1	7ml	11m mol/L Urea hydrogen peroxide
Color Developing Reagent B	1	7ml	2m mol/L 3,3'5,5'-Tetramethylbenzidine
Stop Solution	1	7ml	2mol/L Sulphuric acid
Plate Sealers	2	Piece	

Required Materials That Are Not Supplied

1. Microplate Reader capable of reading absorbance at 450nm.
2. Automated plate washer (optional)
3. Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
4. Deionized or distilled water.
5. 500ml graduated cylinders.
6. Test tubes for dilution.

Typical Data Obtained from β-hCG ELISA Kit

Concentration(IU/L)	0	8	16	40	100	240
O.D.	0.007	0.193	0.337	0.743	1.491	3.152

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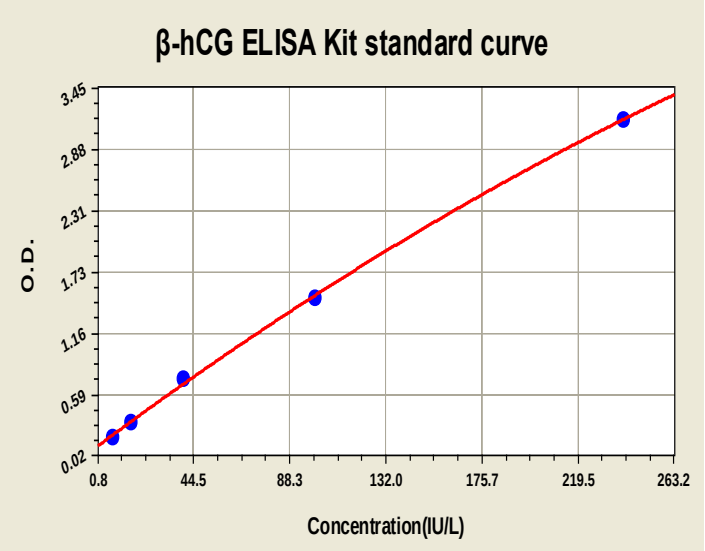

Warnings and Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Sample Preparation and Storage

1. Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
2. Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
   Note: Grossly hemolyzed samples and chylemia samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (20-25°C) for 30 minutes.
20X Wash Buffer Concentrate	Prepare 1X wash buffer by adding 15 ml of Wash Buffer Concentrate to 285 ml deionized or distilled water to prepare 300 mL of Wash Buffer.

10 X Urine Sample Dilute Buffer Concentrate	Prepare 1X dilute buffer by adding 15 ml of Urine Sample Dilute Buffer Concentrate to 135 ml 0.9% NaCl solution to prepare 150 mL of Dilute Buffer.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Set Standard wells, Sample wells, Control wells and Blank wells, add 50 µl of the standard, sample, or control per well. At least two replicates of each standard, sample, control or blank is recommended.
4. Add 50 µl of HRP Conjugated anti-hCG-β antibody to each well except for the blank well and mix thoroughly.
5. Cover with plate sealer and incubate for 60 minutes at 37°C.
6. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
7. Add 50µl Color Developing Reagent A and 50µl Color Developing Reagent B to each well and incubate in the dark for 15 minutes at 37°C.
8. Add 50 µl of Stop Solution to each well.
9. Read absorbance on Plate Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average blank O.D. reading.

It is unnecessary to set blank control for dual wavelength plate reader.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic- curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.