ab285351 – Human Chorionic Gonadotropin (hCG) ELISA Kit

For Quantitative protein detection, establishing normal range etc.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285351

Materials Supplied

Item	Quantity	Storage Condition
Plate coated with hCG MAb, 96 wells	8 wells ×12 strips	4°C
hCG Standard	6 x 500 µl	4°C
hCG Enzyme Conjugate	12 mL	4°C
Wash Concentrate (20X)	25 mL	4°C
TMB Substrate	12 mL	4°C
Stop Solution	12 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

Storage conditions and Reagent Preparation

Store kit at 2-8C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

Wash Concentrate: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

Standards: These are ready to use. Standard 1 (0 mIU/ml), Standard 2 (10 mIU/ml), Standard 3 (25 mIU/ml), Standard 4 (50 mIU/ml), Standard 5 (100 mIU/ml), Standard 6 (250 mIU/ml).

Warnings and Precautions

- 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 Centres for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- This test kit is USA FDA exempt product.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control, and serum samples be run in duplicate.
- Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Sample Preparation

- Collect blood specimens and separate the serum immediately.
- 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.
- Avoid multiple freeze-thaw cycles.
- Prior to assay, frozen sera should be completely thawed and mixed well.
- Do not use grossly lipemic specimens.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Assay Protocol

- Prior to assay, allow reagents to stand at room temperature.
- Gently mix all reagents before use.
- Check hCG standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of hCG standards, control, and sample into designated wells.
3. Add 100 µl of hCG enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 min. at room temperature (18-26° C).
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels
6. Add 100 µl of TMB substrate to all wells & incubate for 10 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stopping solution

Calculation

Construct the standard curve, plot the absorbance for the hCG standards (vertical axis) versus the hCG standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Value above the highest point of the standard are retested after diluting with "0" standard.

Expected Values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for hCG may be used as initial guideline ranges only: hCG Normal Range = Less than 5 mIU/ml.

Technical Support

For all technical or commercial enquiries please go to:

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