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Datasheet for ABIN996905

HCG beta ELISA Kit



Overview

Quantity:	96 tests
Target:	HCG beta
Binding Specificity:	free
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0-50 mIU/mL
Minimum Detection Limit:	0 mIU/mL
Application:	ELISA
Product Details	
Purpose:	free beta hCG-subunit quantitative assay is designed for in vitro quantitative measurement of
	human chorionic gonadotropin free beta-subunit in patient's serum
Sample Type:	Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	100%
Sensitivity:	0.5 mIU/mL
Material not included:	1. Precision pipettes: 0.04 till approx. 0.4 mL
	2. Disposable pipette tips
	3. Distilled water

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- 4. Vortex mixer or equivalent
- 5. Absorbent paper or paper towel
- 6. Graph paper
- 7. Microtiter well reader: A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

Target Details

Target:	HCG beta
Alternative Name:	beta hCG (HCG beta Products)
Background:	Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit. In the normal second-trimester maternal sera, the level of intact hCG range from 20,000 mIU/mI to 50,000 mIU/mI. In contrast, the levels of either free beta- or free beta-hCG are on average one half of 1% of hCG levels. hCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors, however, the absolute increase of beta-hCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy. Recent studies showed a significant increase in the level of free beta-hCG subunit in trisomy 21 cases as compared with controls. Hence, it has been suggested that free beta-hCG subunit assay in a combination of maternal serum AFP
	could be effective in a screening protocol for trisomy 21.

Application Details

Comment:	This kit is for use with serum samples without additives only.
Sample Volume:	20 μL
Assay Time:	1 - 2 h
Plate:	Pre-coated
Reagent Preparation:	1. All reagent should be brought to room temperature (18-22 °C) before use.
	2. Reconstitute each lyophilized standard with 0.4 mL distilled water. Allow the reconstituted
	material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-

- 8 °C, and it will be stable for at least two weeks at that conditions.
- 3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer Concentrate (50x) into distilled water to prepare 750 mL of washing buffer(1x). Mix well before use.

Sample Collection:

Specimen collection and preparation Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques.

Assay Procedure:

- 1. Secure the desired number of coated wells in the holder.
- 2. Dispense 50 mL of standard, specimens, and controls into appropriate wells.
- 3. Dispense 100 mL of Zero Buffer into each well.
- 4. Thoroughly mix for 10 seconds. It is very important to have completed mixing in this setup.
- 5. Incubate at 37 °C for 30 minutes.
- 6. Remove the incubation mixture by flicking plate content into a sink.
- 7. Rinse and empty the microtiter plate 4 times with washing buffer(1X) and 1 time with distilled water.
- 8. Strike the wells sharply onto absorbent paper or paper towels to remov all residual water droplets.
- 9. Dispense 150 mL of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
- 10. Incubate at 37 °C for 30 minutes. Remove the incubation mixture by flicking plate contents into sink.
- 11. Rinse and empty the microtiter plate 4 times with washing buffer(1X and 1 time with distilled water.
- 12. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- 13. Dispense 100 mL TMB solution into each well. Gently mix for 5 seconds.
- 14. Incubate at room temperature in the dark for 20 minutes.
- 15. Stop the reaction by adding 100 mL of Stop Solution to each well.
- 16. Gently mix for 4 till approx. 30 seconds. It is very important to make sure that the blue color changes to yellow color completely.
- 17. Read optical density at 450 nm with a microtiter plate reader within 30 minutes.

Important Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

Limitations and Precaution: The free beta hCG-subunit quantitative assay is designed for in vitro use only. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Calculation of Results:

The absorbance of this quantitative assay is to 50 mIU/mL of beta-hCG. It is recommended

that samples falling above the range should be diluted with sample diluent (Zero Buffer) to absorbance that within the standard curve range. Calculation of results Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/mL on log-log graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of beta-hCG in mIU/mL from the standard curve. Example of standard curve Results of typical standard run with optical density reading at 450 nm shown in the Y axis against hCG concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Expected Values and Indications for Quantitative Free beta-hCG Assay:

- 1. In early pregnancy, free beta-hCG concentration was found 14 till approx. 80 mIU/mL. The free beta-hCG /intact hCG ratio was found 0.44 till approx. '0.48 percents. After 6 to 7 weeks the free beta-hCG and the ratio value declined. During the second and third trimester, a constant ratio was observed about 1 percent.
- 2. Serum samples from 40 normal subjects were assayed, in this population, 99 % of the values were less than 0.4 mlU/mL.

Restrictions:

For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Storage of test kits and instrumentation
	1. Unopened test kits should be stored at 2-8 °C upon receipt and the microtiter plate should be
	kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be
	used throughout the expiration date of the kit (One year from the date of manufacture). Refer to
	the package label for the expiration date.
	2. Opened test kits will remain stable until the expiring date shown.
Expiry Date:	12-14 months