ab178633 – Chorionic Gonadotropin beta Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Chorionic Gonadotropin beta in Human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 5 December 2013

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1. BACKGROUND

Abcam's Chorionic Gonadotropin beta *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Chorionic Gonadotropin beta in Human serum and plasma.

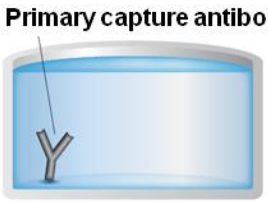
A 96-well plate has been precoated with anti-Chorionic Gonadotropin beta IgG antibodies. Samples, standards and the Chorionic Gonadotropin beta-HRP conjugate are added to the wells, where Chorionic Gonadotropin beta in the sample and standards binds to the precoated antibody and added Chorionic Gonadotropin beta-HRP conjugate binds to this antibody- Chorionic Gonadotropin beta complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Chorionic Gonadotropin beta in the sample and the intensity is measured at 450 nm.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted in pregnancy, that is made by the embryo soon after conception and later by the syncytiotrophoblast (part of the placenta). Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for a pregnancy in humans. hCG may have additional functions, for instance it is thought that it affects the immune tolerance of the pregnancy.

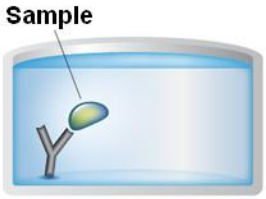
Pregnancy tests measure the levels of hCG in the blood or urine to indicate the presence or absence of an implanted embryo. In particular, pregnancy tests employ an antibody that is specific to the β-subunit of hCG (βhCG). This is important so that tests do not make false positives by confusing hCG with LH and FSH.

βhCG is also secreted by some cancers including teratomas, choriocarcinomas. But, elevated levels cannot prove the presence of a tumor, and low levels do not rule it out.

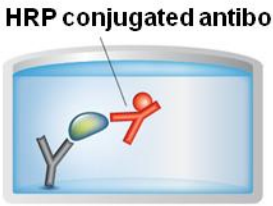
2. ASSAY SUMMARY



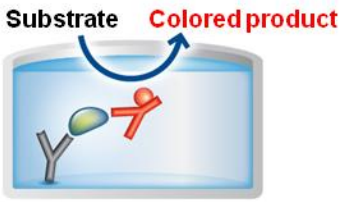
Prepare all reagents, samples and standards as instructed.



Add samples and standards to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Chorionic Gonadotropin beta Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
10X Anti-Chorionic Gonadotropin beta HRP Conjugate	1 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Incubation Buffer	50 mL	2-8°C
50X Washing Solution	20 mL	2-8°C
Chorionic Gonadotrophin beta Control	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 0 – 0 mU/mL	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 1 – 1 mU/mL	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 2 – 5 mU/mL	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 3 – 20 mU/mL	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 4 – 100 mU/mL	1 mL	2-8°C
Chorionic Gonadotropin beta Standard 5 – 400 mU/mL	1 mL	2-8°C
Strip Holder	1 unit	2-8°C
Cover Foil	1 unit	2-8°C

6. 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 or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer
- Absorbent paper or paper towel.

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 490 mL deionized water. Mix thoroughly and gently.

9.2 **1X Anti-Chorionic Gonadotropin beta HRP Conjugate**

Prepare 1X Anti-Chorionic Gonadotropin beta HRP Conjugate by diluting 10X Anti-Chorionic Gonadotropin beta HRP Conjugate with Incubation Buffer. To make 1X Anti-Chorionic Gonadotropin beta HRP Conjugate combine 10 µL 10X Anti-Chorionic Gonadotropin beta HRP Conjugate with 90 µL Incubation Buffer. Mix thoroughly and gently. Stable for 3 hours at room temperature.

- All other solutions are supplied ready to use.

10. SAMPLE COLLECTION AND STORAGE

- Use Human serum or plasma samples with this assay. If the assay is performed within 48 hours after sample collection, the specimens should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -80°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
- Samples with concentration of Chorionic Gonadotrophin beta over 400 mU/mL have to be diluted with Incubation buffer.

Avoid repeated freezing and thawing

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Add 25 µL standards, control and samples into their respective wells.

12.3. Add 100 µL of 1X Anti-Chorionic Gonadotropin beta HRP Conjugate into each well.

12.4. Cover wells with the foil supplied in the kit and incubate at room temperature for 1 hour.

12.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. During each washing step, gently shake the plate for 5 sec and remove excess solution by tapping the inverted plate on an absorbing paper towel. After the last wash, remove the remaining deionized or distilled water by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

12.6. Add 100 µL TMB Substrate Solution into all wells.

12.7. Incubate at room temperature for 15 minutes in the dark.

12.8. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Note: Any blue color developed during the incubation turns into yellow.

12.9. Read the absorbance at 450 nm against a reference wavelength of 620 – 630 nm or against a blank within 5 minutes of addition of the Stop Solution.

13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

14. TYPICAL SAMPLE VALUES

REFERENCE VALUES –

Each laboratory must establish its own normal ranges based on patient population.

The serum or plasma Chorionic Gonadotropin beta values are comprised in the following intervals:

Normal women:		< 8 mU/mL
Pregnancy:	**Week 1**	3 - 100 mU/mL
	Week 2	10 - 1,000 mU/mL
	Week 3	100 - 10,000 mU/mL
	Week 4	1,000 - 100,000 mU/mL
	Month 2	15,000 - 200,000 mU/mL
	Month 3	10,000 - 100,000 mU/mL

PRECISION –

	Intra-Assay	Inter-Assay
n=	48	3
%CV	≤ 7.6	≤ 8.8

SENSITIVITY –

The minimum detectable concentration of Chorionic Gonadotropin beta from Standard 0 is estimated to be 0.09 mU/mL.

ACCURACY –

The recovery of 6.25 – 12.5 – 25 – 50 mIU/ml of Chorionic Gonadotropin beta added to sample gave an average value (± SD) of 99.2 % ± 4.1% with reference to the original concentrations.

15. ASSAY SPECIFICITY

The cross reactivity values of the β-HCG ELISA have been calculated on a weight/ weight basis.

b-HCG	100.00%
hFSH	3.00 %
HCG	4.00 %
hTSH	0.02 %

16. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

17. NOTES

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