



Chorionic Gonadotropin (hCG) (human) ELISA Kit

10/14

(Catalog # K7424-100, 100 assays; Store at 2-8°C)

I. Introduction:

Human Chorionic Gonadotropin (hCG) is a 40 kD glycoprotein hormone secreted by the placenta. The serum hCG rises in early pregnancy to concentrations of 50,000-150,000 mIU/ml between the 8th and 12th weeks of gestation and decline to 20,000 mIU/ml by the 18th week where they remain for the duration of the pregnancy. The increased level of hCG in non-pregnant women or men suggest neoplasia. Thus hCG measurement is useful for the recognition and monitoring of chorionic tumors and as a tumor marker for other malignancies that produce hCG ectopically. These include testicular, pancreatic, and bronchogenic pulmonary cancers. BioVision hCG ELISA Kit is a direct solid phase sandwich ELISA method. The samples and diluted anti-hCG-HRP conjugate are added to the wells coated with Mab to beta subunit. hCG in the serum binds to anti-hCG MAb on the well and the anti-hCG second antibody then binds to hCG. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of hCG in the samples. A standard curve is prepared relating color intensity to the concentration of the hCG. The sensitivity of this ELISA test is 0.5mIU/ml.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human hCG.

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7424-100	Part No.
Plate coated with hCG MAb, 96 wells	12 stripsx8 wells	K7424-100-1
hCG Standard	0.5 ml x 6	K7424-100-2.x
hCG Enzyme Conjugate	12 ml	K7424-100-3
Wash Concentrate (20X)	25 ml	K7424-100-4
TMB Substrate	12 ml	K7424-100-5
Stop Solution	12 ml	K7424-100-6

VI. User Supplied Reagents and Equipment:

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

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. 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).

VIII. Warning & 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 Centers 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.

IX. Sample Preparation and Storage:

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.

X. 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.

XI. 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.

Example of a Standard Curve:

Standard	OD (450 nm)	Part No.
Standard 1 (0 mIU/ml)	0.048	K7424-100-2.1
Standard 2 (10 mIU/ml)	0.169	K7424-100-2.2
Standard 3 (25 mIU/ml)	0.357	K7424-100-2.3
Standard 4 (50 mIU/ml)	0.650	K7424-100-2.4
Standard 5 (100 mIU/ml)	1.198	K7424-100-2.5
Standard 6 (250 mIU/ml)	2.642	K7424-100-2.6

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

XII. RELATED PRODUCTS:

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| Chorionic Gonadotropin (CG), human (4778) | Growth hormone Antibody (5769) |
| Human CellExp™ HGH, human recombinant (6457) | Growth hormone, chicken recombinant (4771) |
| Growth hormone, human recombinant (4769) | Growth hormone, murine recombinant (4770) |
| Chicken Growth hormone Antibody (6648) | |

FOR RESEARCH USE ONLY! Not to be used on humans.