Human Free beta hCG ELISA Kit

Enzyme Immunoassay for the quantification of free beta subunit of human chorionic gonadotropin (free beta hCG) in serum and plasma.

Catalog number: ARG80785

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INTRODUCTION

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.

The measurement of free β-HCG in the first trimester of pregnancy has been reported as a useful marker in antenatal screening for Down Syndrome and other fetal aneuploidies. Increased free β-HCG values in combination with maternal age, the measurement of PAPP-A and the ultrasonic determination of nuchal translucency (NT) in pregnancy weeks 11 to 14 may detect up to 90 % of pregnancies with Down syndrom.

The Free β-HCG ELISA ARG80785 may be used for the risk assessment of Down´s syndrom (trisomy 21) in the first trimester of pregnancy. For the risk assessment of trisomy 21 and other fetal aneuploidies free beta HCG should always be measured in combination with other analytes (for example PAPP-A and NT, see above) and a special software for the risk assessment of trisomy 21. According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of free beta subunit of human chorionic gonadotropin (free beta-HCG) in serum and plasma.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody for Human Chorionic Gonadotropin (HCG) molecule has been pre-coated onto a microtiter plate. Standards, controls or samples are pipetted into the wells and any free HCG present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-labeled HCG antibody is added to each well and incubate. A substrate solution (TMB) is then added to the wells and color develops in proportion to the amount of HCG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of HCG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Controls	2 vials (Lyophilized)	4°C.
Standards 0-5	6 vials (Lyophilized) (0,10,25,50,100,200 ng/ml)	4°C

Zero Buffer	14ml (Ready-to-use)	4°C
HRP antibody conjugate	18ml (Ready-to-use)	4°C
Diluent for HRP antibody conjugate	14ml (Ready-to-use)	4°C
40X Wash buffer	30ml	4°C
TMB substrate	14ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. Diluted wash buffer is stable for 2 weeks at RT.
- Controls: Reconstitute lyophilized controls in 1ml distilled water. Stable for 1 month at 2-8°C. For longer storage, freeze at -20°C.
- Standards: Reconstitute lyophilized standards in 1ml distilled water. Stable for 1 month at 2-8°C. For longer storage, freeze at -20°C.
- Sample dilution: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with samples diluent and re-assay.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

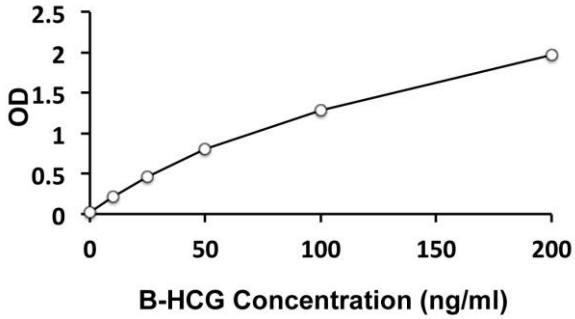
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of controls, standards, samples and blank into respective wells.
3. Add 100 µl of zero buffer into each well.
4. Cover wells and incubate for 30 mins at 37°C.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 150 µl HRP antibody conjugate into each well. Incubate for 30 minutes at 37°C.
7. Wash as according to step 5.
8. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature (in dark).
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of total bHCG ranged from 10-200ng/ml.

The mean MDD was 0.2ng/ml.

Specificity

No interference has been observed with the following factors:

TSH, FSH, Prolactin, LH

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.4% and inter-assay precision was 7.58%.

Recovery

93.7—99.4%

Linearity

92.9—113%