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Product Information

Anti-Chorionic Gonadotropin (α + β subunits) (HCG) produced in rabbit, whole antiserum

Catalog Number **C8534**

Product Description
The antiserum is produced in rabbit using as immunogen human chorionic gonadotropin (HCG).The product has been evaluated for use in a radio-immunoassay (RIA) system, hemagglutination inhibition assay and in immunohistology.

Reagents
Supplied as an undiluted antiserum containing 0.1% sodium azide as a preservative.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

RIA System
RIA Characterization
The antiserum is characterized utilizing the following second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1 ml of antiserum at the working dilution binds at least 40% of 200 picograms of iodinated HCG with a specific activity of ~100μCi/μg.

Note: It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in systems and procedures.

RIA Dilution Instructions
A minimum working dilution of 1:50,000 was determined by second antibody-polyethylene glycol (PEG) RIA. Dilute the antiserum in 0.01 M phosphate buffered saline, pH 7.8, containing 0.5% BSA and 0.1% sodium azide.

RIA Reagents
1. Standards: Prepare and freeze aliquots of a stock standard solution of 10 IU/ml HCG, Catalog Number C2047; immunopotency ~14,000 IU/ml, in dilution buffer. Thaw one aliquot for each assay and dilute in dilution buffer to the following concentrations: 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mIU/ml.
2. Dilution Buffer: 0.01 M PBS, pH 7.8, containing 0.5% BSA and 0.1% sodium azide.
3. Rabbit Serum, Catalog Number R9133, 2% in dilution buffer without BSA.
4. EDTA solution: EDTA disodium salt, Catalog Number ED2SS, 0.1 M, pH 7.8, in distilled water. Adjust pH with 10 N NaOH.
5. Second Antibody: Anti-Rabbit IgG (whole molecule), Catalog Number R0881, reconstituted in dilution buffer. Dilute reconstituted antiserum in dilution buffer as recommended.
6. PEG solution: 6% PEG, Catalog Number P2139, average mol wt 8,000, in dilution buffer without BSA.

RIA Protocol
1. In polypropylene test tubes add 0.2 ml sample or standard and 0.1 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 1 hour at 37 °C.
4. Add 0.1 ml I^{125} radioactive tracer diluted in dilution buffer.
5. Vortex the tubes.
6. Incubate for 2 hours at 37 °C followed by an incubation of 18-20 hours at 4 °C.
7. Add 0.1 ml EDTA solution and 0.1 ml 2% rabbit serum to all tubes.
8. Vortex the tubes.
9. Add 0.1 ml second antibody to all tubes.
10. Add 0.5 ml PEG solution to all tubes.
11. Vortex the tubes.
12. Incubate for 5 minutes at room temperature.
13. Centrifuge at 2000 x *g* for 15 minutes at 4 °C.
14. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

RIA Specificity
Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the second antibody-PEG I^{125} RIA system is as follows:

Cross-Reactant	**%Cross-Reactivity**
Human Follicle Stimulating Hormone (HFSH)	< 5
Human Growth Hormone (HGH)	< 5
Human Luteininzing Hormone (HLH)	> 11.0
Human Thyroid Stimulating Hormone (HTSH)	< 20

RIA Sensitivity
Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be at least 0.2 mIU HCG/tube.

RIA Affinity Constant
The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.
$K_a = 2 \times 10^{10}$ to 2×10^{11} L/M.

Immunohistology

Specificity
Staining of the syncytiotrophoblast and cytotrophoblast layers of the placental villi is observed, while other placental elements and non-endocrine organs are not stained. Due to cross-reactivity with LH, FSH and TSH (through a common α-chain) staining of the pituitary gland can also be demonstrated.

Working Dilution
The working dilution was determined to be 1:100-1:200 by indirect immunoperoxidase staining of human placental tissue.

Note: In order to obtain best results, it is recommended that each individual user determine their working dilutions by titration assay.

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