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ProductInformation

Monoclonal Anti-β-Human Chorionic Gonadotropin (βhCG)
Clone: PC-2
Mouse ascites fluid

Product Number **C 7659**

Product Description

Monoclonal Anti-β-Human Chorionic Gonadotropin (βhCG) (mouse isotype IgG1) is derived from hybridoma PC-2, created from fusion between a mouse myeloma cell line (NS1) and splenocytes from BALB/c mice immunized with purified hCG.

Reagent

Monoclonal Anti-β-Human Chorionic Gonadotropin (βhCG) is presented in the form of a diluted ascites fluid obtained from BALB/c mice bearing the PC-2 hybridoma.

Storage/Stability

For continuous use, store at 2-8 °C. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Antibody Performance in RIA System:
1. Double antibody RIA (see attached procedure).
2. Tracer: Iodine125-hCG. Specific activity of approx. 80 µCi/ug.

Titer:
1:8,000-1:12,000 dilution will bind approximately 25-35% of 200 pg 125I-hCG.

Sensitivity:
Can detect 1 ng (10 mIU/ml)

Specificity:

hCG	100%	A typical dose response
HLH	< 0.2%	and cross reactivity
HFSH	< 0.1%	curves is enclosed.
HTSH	< 0.1%	

Antibody Affinity Constant Ka: 3.0×10^{10} L/M (SCATCHARD PLOT)

Double Antibody RIA for Monoclonal β-hCG

Reagents
1. Buffer: 0.01M phosphate buffer (pH 7.8) containing 0.15M NaCl, 0.5% bovine serum albumin (BSA) and 0.1% NaN_3.
2. Standards: Prepare a concentrated stock solution of hCG in buffer at 1 µg (10 IU)/ml. Distribute into small aliquots and thaw only one aliquot for each assay. Dilute in buffer to obtain the following concentrations:

125.0 mIU/ml	15.6 mIU/ml
62.5 mIU/ml	7.8 mIU/ml
31.2 mIU/ml	3.9 mIU/ml

3. Anti-βhCG: Dilute the Monoclonal Anti-βhCG with buffer to the titer specified in each lot.
4. Radiolabelled Tracer: Freshly prepared ^{125}I-hCG (specific activity approximately 80 µCi/µg) can be used as it is. One week or older preparations should be purified prior to use. (A Sephadex G-75 column equilibrated with the above described buffer can be used for such a purification). Dilute the ^{125}I-hCG solution to a concentration of 2.0 ng/ml with buffer.
5. EDTA Solution: 0.1M ethylenediaminetetraacetic acid disodium salt (EDTA) in distilled water, pH adjusted to 7.8 with 10M NaOH.
6. Normal Mouse Serum: On the second day of the test dilute normal mouse serum with buffer (without BSA) to a concentration of 2% (v/v).
7. Second Antibody: Rabbit Anti-Mouse IgG (Sigma Code 1165) is used. Reconstitute to a final concentration of 1 mg/ml with buffer.
8. Polyethylene Glycol (M.W. approximately 6000): Prepare a stock solution of 60 g/liter in buffer (without BSA).

Method:
1. Pipette 0.2 ml of hCG standards to assay tubes. Prepare a zero control and a blank tube each containing 0.2 ml of buffer.
2. Add 0.1 ml of antibody to all tubes except the blank tube. To this, add 0.1 ml buffer.

3. Incubate all tubes at 37 °C for 1 hour.
4. Prepare at least two empty tubes for the total count. After the incubation period (step 6) put them aside; count with the rest of the tubes (step 12).
5. Add 0.1 ml of ^{125}I-hCG solution to all tubes. Incubate at 37 °C for 2 hours.
6. Incubate at 2-8 °C overnight (18-20 hours).
7. Add 0.1 ml of EDTA solution and 0.1 ml of diluted normal mouse serum to all tubes. Mix.
8. Add 0.1 ml of second antibody to all tubes. Mix and incubate at 2-8 °C for 30 minutes.
9. Add 0.7 ml PEG to each tube (except the total). Mix well by vortex.
10. Centrifuge at 3000 rpm at 2-8 °C for 15 minutes.
11. Carefully aspirate off the supernatants.
12. Count the precipitate in a gamma counter.

Calculations:

1. Convert all counts to counts per minute (cpm).
2. For each sample or standard tube calculate the % of zero bound (B/Bo) as follows:

$$\%\ B/Bo = \frac{\text{cpm in sample - cpm in blank}}{\text{cpm in zero control - cpm in blank}} \times 100$$

3. Generate a standard curve by plotting the % B/Bo against the log dose of standard.
4. Determine the concentration of hormone in each sample by interpolation from the standard curve.

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