prima

Technical Bulletin

Monoclonal Anti-Progesterone

Clone 2H4 Rat Cell Culture Supernatant

P1922

Storage Temperature –20 °C

Product Description

Progesterone or P4 (pregn-4-ene-3,20-dione) is a female steroid hormone that belongs to the hormones class, progestogens. It is produced majorly by ovaries, adrenal glands and placenta of humans and other species. Progesterone derived from placenta and corpus luteum is required for the maintenance of pregnancy. Progesterone inhibits the T cell mediated immune response involved in tissue rejection. The progesterone levels in females are low before ovulation and are elevated during the luteal phase[2,3]. Monoclonal Anti-Progesterone antibody is IgG1 isotype purified from rat cell culture supernatant.

Reagent

The product is provided as the cell culture supernatant with 0.1% sodium azide as a preservative.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store undiluted product at –20 °C in working aliquots. Repeated freezing and thawing is not recommended. Do not store in a frost-free freezer.

Working dilution samples should be discarded if not used within 12 hours.

Procedure

The binding activity and subsequent lot specific data presented in the Certificate of Analysis indicate the performance of the antibody in the assay system. It is recommended that the antibody first be evaluated in the particular assay system chosen, due to differences in assay systems and procedures.

Reagents

- Buffer: 0.05 M Tris-HCl, pH 8.0, with 0.1M NaCl, 0.1% gelatin, and 0.1% sodium azide.
- Dextran-Coated Charcoal: 0.5% (w/v) Dextran T-70 in buffer without gelatin. Mix for 1 hour at 4 °C prior to use.
- Standards: Prepare a standard solution of 1 µg/mL of progesterone in absolute ethanol. Dilute an aliquot in buffer to a concentration of 5 ng/mL. Six further serial doubling dilutions are prepared in buffer from the 5 ng/mL standard giving the following concentrations: 2.5, 1.25, 0.63, 0.31, 0.15, and 0.078 ng/mL.
- Radiolabeled progesterone: Prepare a fresh solution of 70,000-100,000 dpm/mL of tritiated progesterone.

RIA Protocol

1. The working dilution of the product is prepared with 0.05 M Tris-HCl buffer, pH 8.0, with 0.1 M NaCl, 0.1% gelatin and 0.1% sodium azide.
2. In polypropylene test tubes add 0.1 mL of sample or standard and 0.5 mL of monoclonal antibody, diluted to working dilution, and mix.
3. Incubate tubes at room temperature for 30 minutes.
4. Add 0.1 mL of tritiated progesterone to all tubes and mix. Incubate at 37 °C for 60 minutes.
5. Cool at 4 °C for 15 minutes.
6. Add 0.2 mL of dextran coated charcoal solution to each tube, excluding the total tube to which 0.2 mL of buffer is added.
7. Vortex all tubes and incubate at 4 °C for 10 minutes. Centrifuge at 4 °C for 15 minutes at 3000 rpm.
8. Remove 0.25 mL of the supernatant and add 3 mL of scintillation fluid. Determine the amount of radioactivity present.

Product Profile

Monoclonal anti-Progesterone may be used in various immunochemical techniques including ELISA, radioimmunoassay and RIA to measure progesterone levels in milk.[1] Working dilution of 1:1000-1:2000 was determined using indirect ELISA and Progesterone-BSA for coating.

References

1. J. S. Mitchell et. Al J Dairy Sci. 2004 Sep;87(9):2864-7.
2. P K Siiteri et. al. Annals of the New York Academy of Sciences 1977-3-11.
3. N Applezweig Chemical week, 104, undefined (1969-5-17).

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