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ProductInformation

Anti-Rabbit IgG (whole molecule)
Developed in Goat
Whole Antiserum

Product No. **R 0881**

Product Description
The antiserum is developed in goat using rabbit IgG as the immunogen. The antiserum is tested for specificity by immunoelectrophoresis (IEP) and selected on the basis of titer and minimum cross-reactivity with human serum. It has been characterized as a second antibody for use in a radioimmunoassay (RIA).

Reagent
The antibody is provided as lyophilized whole serum with no preservatives.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

This goat antiserum was maintained at pH 5.0 for 40 minutes to meet U.S.D.A. requirements.

Reconstitution Instructions
Reconstitute the contents of the vial with 5 ml of buffer (0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide). Rotate the vial gently until the powder dissolves. This is the stock antiserum solution. To obtain the number of tests (working dilution) indicated on the vial dilute the reconstituted antiserum as appropriate for the package size in the same buffer that was used to reconstitute the antiserum.

Dilute the 250 test package size 1:5 to obtain 250 tests.
Dilute the 1,000 test package size 1:20 to obtain 1,000 tests.
Dilute the 5,000 test package size 1:100 to obtain 5,000 tests.

The working dilutions and buffer used will vary with the protocol chosen for the assay. The recommended dilution is intended only as a guide and represents the working dilution of the anti-rabbit IgG antiserum required for maximum precipitation in an RIA procedure for human chorionic gonadotropin (HCG) using 4 µl of carrier normal rabbit serum.

Storage
Prior to reconstitution, store the antiserum at 2-8 °C. After reconstitution the stock solution should be separated into aliquots and frozen. Repeated freezing and thawing is not recommended.

Tests per Vial
The precipitation technique that follows can be used in assays for low or high molecular weight antigens. The number of tests per vial is determined utilizing the following typical second antibody-polyethylene glycol (PEG) RIA protocol for HCG. When reconstituted and diluted according to the above instructions, one test provides an adequate amount of antiserum to precipitate the maximum amount of IgG using the protocol for HCG.
It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in systems and procedures.

Reagents for Use with Second Antibody
In addition to the reagents needed for the antigen (primary antibody reaction), prepare the following reagents for the precipitation step:

(A) Normal rabbit serum (Product No. R 9133), 2.0% in assay buffer without BSA.
(B) EDTA Solution: 0.1 M ethylenediamine-tetraacetic acid (EDTA) disodium salt (Product No. E 2501) in distilled water. Adjust pH to that of the buffer being used.
(C) PEG Solution: 6% PEG (Product No. P 2139, approximate molecular weight 8,000) in assay buffer without BSA.

RIA Protocol for HCG Excluding Precipitation Step

1. In polypropylene test tubes add 0.2 ml sample or standard and 0.1 ml diluted Rabbit Anti-HCG antiserum.
2. Vortex the tubes.
3. Incubate for 1 hour at 37 °C.
4. Add 0.1 ml ^{125}I-HCG tracer.
5. Vortex the tubes.
6. Incubate for 2 hours at 37 °C, followed 18-20 hours incubation at 2-8 °C.

RIA Protocol for Precipitation Step

1. Upon completion of the second incubation step (step 6) add 0.1 ml EDTA solution (B) and 0.2 ml 2.0% normal rabbit serum (A).
2. Vortex the tubes.
3. Add 0.1 ml Goat Anti-Rabbit IgG antiserum at the working dilution.
4. Vortex the tubes.
5. Add 0.7 ml PEG solution (C) to all tubes.
6. Vortex the tubes.
7. Incubate for 5 minutes at room temperature.
8. Centrifuge at 2000 x *g* for 15 minutes at 2-8 °C.
9. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

Bibliography

1. Hunter, W., In: Methods of Hormone Analysis, Breuer, H., et al., (Eds.), John Wiley and Sons, New York, p. 3 (1976).
2. Tyrey, L., et al., In: Methods of Hormone Radioimmunoassay, Jaffe, B. and Behrman, H., (Eds.), Academic Press, New York, p. 427 (1974).
3. Wood, W., et al., J. Clin. Chem. Clin. Biochem., **17**, 111 (1979).

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