



## Product Information

### Anti-Luteinizing Hormone Releasing Hormone (LHRH) Developed in Rabbit Whole Antiserum

Product No. L 8391

The antiserum is developed in rabbit using luteinizing hormone releasing hormone-BSA (LHRH-BSA) as the immunogen. The product is provided as diluted antiserum that has been lyophilized.\*

#### Reconstitution and Storage Instructions

To one vial of lyophilized antiserum, add 1.0 ml of 0.01 M PBS, pH 7.4, containing 0.1% sodium azide. Rotate gently until powder dissolves, this is the stock antiserum solution. To obtain the number of tests indicated on the vial (working dilution), the reconstituted antiserum should be further diluted (1:200) with the same buffer used to reconstitute the antiserum.

Prior to reconstitution, store at 2-8 °C. After reconstitution, the stock solution should be aliquoted and frozen. If antiserum diluted to the working dilution is unused within 12 hours it should be discarded. Repeated freezing and thawing is not recommended.

#### RIA SYSTEM

##### RIA Characterization

The antiserum is characterized utilizing the following second antibody-polyethylene glycol (PEG) RIA protocol, where 0.2 ml of antiserum at the working dilution has been found to bind at least 40% of 50-100 picograms of iodinated LHRH with a specific activity of approximately 100-300  $\mu\text{Ci}/\mu\text{g}$ .

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

##### RIA Reagents

- Standards: Prepare a stock standard solution of 1 mg/ml LHRF (Product No. L 7134) in dilution buffer. Dilute a portion of the stock solution to 20 ng/ml in dilution buffer, this is then further diluted to give standard solutions of the following concentrations: 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 ng/ml.

- Dilution buffer: 0.01 M phosphate buffered saline, pH 7.4, containing and 0.1% sodium azide.
- Normal rabbit serum (NRS, Product No. R 9133), 2% in dilution buffer.
- EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS), 0.1 M, pH 7.5 in distilled water. Adjust pH with 10 N NaOH.
- Second antibody: Goat Anti-Rabbit IgG (Product No. R 0881), reconstituted in dilution buffer. Dilute reconstituted antiserum 1:5 in dilution buffer for use.

##### RIA Protocol

- In polypropylene test tubes, add 0.5 ml sample or standard, 0.2 ml diluted antiserum and 0.1 ml  $I^{125}$  radioactive tracer diluted in dilution buffer.
- Vortex the tubes.
- Incubate for 4 hours at 37 °C.
- Add 0.2 ml 2.0% NRS to each tube.
- Vortex the tubes.
- Add 0.1 ml EDTA solution to all tubes.
- Vortex the tubes.
- Add 0.1 ml second antibody to all tubes.
- Vortex the tubes.
- Incubate for 18-20 hours at 4 °C.
- Centrifuge at 2000 x g for 15 minutes at 4 °C.
- 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  $I^{125}$  RIA system is as follows:

<b>Cross-Reactant</b>	<b>%Cross-Reactivity</b>
Human Follicle Stimulating Hormone (HFSH)	<0.1
Human Luteinizing Hormone (HLH)	<0.1
Prolactin	<0.1

#### **RIA Sensitivity**

Sensitivity is defined as the 90% intercept of a B/B<sub>0</sub> standard curve. In the above system the sensitivity has been found to be 50pg/tube.

#### **RIA Affinity Constant**

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using this RIA system.

K<sub>a</sub> = 1-10 x10<sup>9</sup> L/mole.

#### **Bibliography**

Koch, Y., et al., Biochem. Biophys. Res. Comm., **55**, 616 (1970).

\*Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP 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.

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