ANTI-HUMAN THYROID STIMULATING HORMONE (TSH)
Developed in Rabbit
Whole Antiserum

Product No. T 4651

Product Description
The antiserum is developed in rabbit using human TSH as the immunogen.

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 has been found to bind at least 40% of 75 picograms of iodinated TSH with a specific activity of approximately 70 µCi/µg. 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
The working dilution is determined to be 1:1,000, dilute the antiserum in 0.01 M phosphate buffer saline, pH 7.4, containing 2.0% rabbit serum and 0.1% sodium azide.

RIA Reagents
1. Standards: Prepare a solution of 1 mIU/ml HTSH (Product No. T 0145; immunopotency approximately 5-8 IU/mg by RIA vs. WHO standard 68/38) in dilution buffer. Dilute in same buffer to obtain the following concentrations: 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, and 0.95 mIU/0.1 ml.
2. Buffer: 0.01 M phosphate buffered saline, pH 7.4 containing 2%. BSA (Product No. A 7030) and 0.1% sodium azide.
3. Assay buffer: Normal rabbit serum (Product No. R 9133) 2% in dilution buffer without BSA.
4. Radiolabeled tracer: Freshly prepare a solution of 0.75ng/ml I^{125}-TSH in assay buffer using I^{125}-TSH with a specific activity of approx. 70µCi/µg. The solution yields approx. 500,000 cpm/ml.
5. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS), 0.1 M, pH 7.4 in dilution buffer. Adjust pH with 10 N NaOH.
7. PEG solution: 6% PEG (Product No. P 2139, approximate molecular weight 8,000) in dilution buffer without BSA.

RIA Protocol
1. In polypropylene test tubes add 0.1 ml sample or standard, 0.1 ml EDTA solution and 0.1 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 18-20 hours at 4 °C.
4. Add 0.1 ml I^{125} radioactive tracer diluted in rabbit serum buffer (reagent buffer 3).
5. Vortex the tubes.
6. Incubate for 72 hours at 4 °C.
7. Add 0.1 ml second antibody to all tubes.
8. Vortex the tubes.
9. Add 0.5 ml PEG solution to all tubes.
10. Vortex the tubes.
11. Centrifuge at 2000 x g for 15 minutes at 4 °C.
12. 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:

<table>
<thead>
<tr>
<th>Cross-Reactant</th>
<th>%Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Follicle Stimulating Hormone (HFSH)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Human Luteinizing Hormone (HLH)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Human Chorionic Gonadotropin</td>
<td>&lt;0.001</td>
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</tbody>
</table>
RIA Sensitivity
Sensitivity is defined as the 90% intercept of a B/B₀ standard curve. In the above system the sensitivity has been found to the 0.2 µu TSH per tube.

RIA Affinity Constant
The affinity constant (Kₐ) is determined by a Scatchard plot using this RIA system. Kₐ = 1-9 x 10(10) L/mole.

Reagents
The product is provided as an prediluted antiserum containing 0.1% sodium azide as a preservative.

Precautions and Disclaimer
Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP and 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.

Storage
For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen 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.

Reference

Pcs3/00