SIGMA CHEMICAL COMPANY

Product No. G-2894
Anti-Human Growth Hormone
Developed in Rabbit
Whole Antiserum

Lot 79F4805

The antiserum is developed using human growth hormone (HGH) as the immunogen. The product is diluted antiserum that has been lyophilized from a solution containing 0.02% sodium azide as a preservative.*

Reconstitution and Dilution

To one vial of lyophilized powder, add 1.0 ml phosphate buffer (0.01 M, pH 7.4) containing 0.15 M NaCl, 1.0% bovine serum albumin (BSA) and 0.1% sodium azide. Rotate gently until the powder dissolves. This is the stock antiserum solution. To obtain the number of tests indicated on the vial, the reconstituted antiserum should be further diluted 10-fold with the same buffer to produce the working dilution of the antiserum.

Storage

Prior to reconstitution store at 0-5°C. After reconstitution, the stock solution should be stored frozen in working aliquots. An aliquot of diluted antiserum should be discarded if unused within 12 hours. Repeated freezing and thawing is **not** recommended.

Tests Per Vial

The number of tests per vial is determined at Sigma utilizing the following second antibody-polyethylene (PEG) radioimmunoassay (RIA) protocol where 0.1 ml of reconstituted and diluted antiserum has been found to bind at least 30% of 50 picograms of iodinated HGH with a specific activity of approximately 90 µCi/µg.

The number of tests per vial and subsequent lot specific data indicate the performance of the antiserum in the assay system utilized at Sigma. It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in assay systems and procedures.

Reagents

(A) Standard: Prepare a stock standard solution of 1 mg/ml HGH in buffer (B). Dilute a portion of the stock standard with buffer (B) to a concentration of 1000 pg/0.1 ml. The 1000 pg/0.1 ml standard should be further diluted in buffer (B) to obtain standard solutions at the following concentrations: 4, 8, 16, 32, 63, 125, 250, 500, and 1000 pg/0.1 ml.
(B) Phosphate buffer (0.01 M, pH 7.4) containing 0.15 M NaCl, 1.0% BSA (Sigma Product No. A-7030) and 0.1% sodium azide.
(C) EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Sigma Product No. ED2SS) solution (0.1 M, pH 7.6) in distilled water. Adjust pH with 5 N NaOH.
(D) Second antibody: Rabbit IgG (whole molecule) antiserum (Sigma Product No. R-0881) developed in goat for use in RIA as a second antibody. Reconstitute the lyophilized product as recommended with buffer (B) and dilute 1:5 in buffer (B).
(E) Normal rabbit serum (NRS): Freshly prepared 1.0% NRS in buffer (B).
(F) PEG solution: 6% PEG approximate molecular weight 8,000 (Sigma Product No. P-2139) solution in buffer (B).

RIA Protocol

1. In polypropylene test tubes add 0.1 ml sample or standard (A) and 0.1 ml antiserum reconstituted and diluted in buffer (B) to the working dilution.
2. Vortex the tubes.
3. Incubate for 5 hours at 37°C.
4. Add 0.1 ml I-125 radioactive tracer prepared fresh in buffer (B).
5. Vortex the tubes.
6. Incubate for 18-20 hours at 37°C.
7. Add:
   0.1 ml EDTA solution (C),
   0.1 ml 1.0% NRS (E),
   0.1 ml second antibody (D), and
   0.5 ml PEG solution (F).
8. Vortex the tubes.
9. Centrifuge at 2500 x g for 15 minutes at 4°C.
10. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

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 Sigma's second antibody-PEG I-125 RIA system is as follows:

Cross-Reactant	% Cross-Reactivity
Glucagon	<0.1
Human chorionic gonadotropin (HCG)	<0.1
Human placental lactogen	2.0
Thyroid stimulating hormone (TSH)	<0.1
Insulin	<0.1
Luteinizing Hormone (LH)	<0.1
Prolactin	<0.1

Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. Using Sigma's RIA system the sensitivity has been found to be 10 pg per tube.

Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using Sigma's RIA system.
$K_a = 2.8 \times 10^{11}$ L/mole.

Human Growth Hormone LEVELS

Normal Values[1]

Basal normal values of HGH in blood are variably influenced by time of day, meals, sleep-wake cycle, hormonal status, activity or sex of the

subject. After fasting the expected normal ranges are:

Adult male	Undetectable-8 ng/ml
Adult female	Undetectable-30 ng/ml
Children	Undetectable-10 ng/ml

Bibliography

1. Peake, T.T., Methods of Hormone Radioimmunoassay, (Jaffe, B.M. and H.R. Behrman, eds., Academic Press, New York), pp. 103-123 (1974).

* 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.