Rat Growth Hormone ELISA Kit

Catalog Number KRC5311 (96 tests)

Pub. No. MAN0011120 **Rev.** 6.0 (32)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Rat Growth Hormone ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of rat growth hormone in rat serum, plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant rat growth hormone.

Growth hormone (GH), also known as somatotrophin or somatotropin, is a single-chain polypeptide hormone produced and secreted by somatotroph cells within the lateral wings of the anterior pituitary gland. GH affects cell growth, differentiation, metabolism, and the immune system. Circulating level of GH varies between days and individuals, with approximately 50% of circulating GH bound to GH binding protein (GHBP). GH secretion is affected by many physiological factors (e.g., sleep, exercise, blood sugar, glucocorticoids, androgen and estrogen, ghrelin, and circulating concentrations of GH and IGF-1).

Contents and storage

Upon receipt, store the kit at 2–8°C.

Contents	Cat. No. KRC5311 (96 tests)
Rt Growth Hormone Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Rt Growth Hormone Antibody Coated Plate, 96-well plate	1 plate
Rt Growth Hormone Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X)	0.15 mL
Streptavidin-HRP Diluent	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	4

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Orbital microplate shaker set to approximately 400 rpm

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

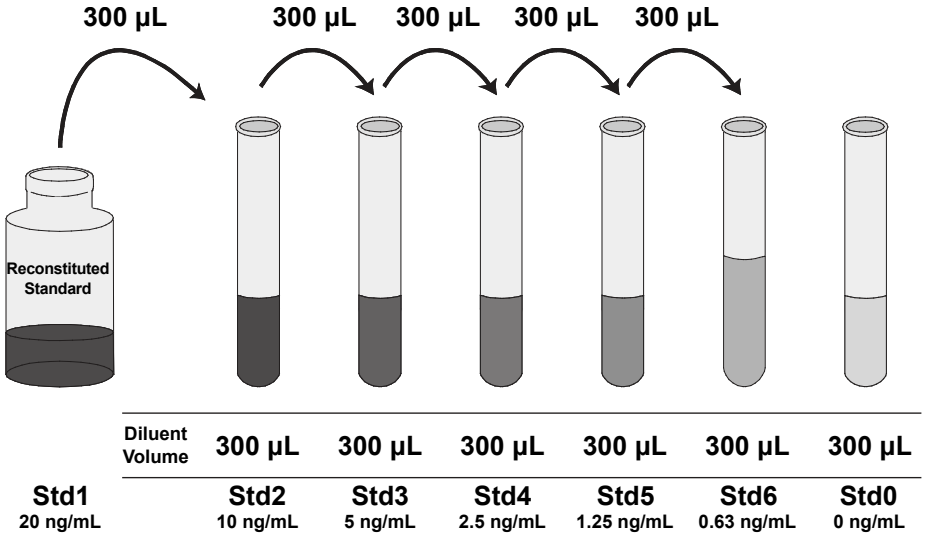
Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute serum, plasma and tissue culture samples 1:5 in Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rt Growth Hormone Standard to 20 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 20 ng/mL rat growth hormone. **Use the standard within 15 minutes of reconstitution.**
2. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.63, and 0 pg/mL rat growth hormone.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, and dispense the solution into a tube containing 1 mL of 1X Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

IMPORTANT! Perform all incubation steps on an orbital microplate shaker (set to approximately 400 rpm).

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Rt Growth Hormone Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

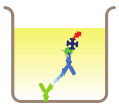
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0 to 20 ng/mL rat growth hormone.

Standard Rat Growth Hormone (ng/mL)	Optical Density (450 nm)
20	3.05
10	1.40
5	0.61
2.5	0.31
1.25	0.17
0.63	0.12
0	0.06

High-dose hook effect

No hook effect was observed with concentrations up to 1 µg/mL.

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	1.62	3.13	10.02
Standard Deviation	0.09	0.19	0.31
% Coefficient of Variation	5.8	6.0	3.1

Intra-assay precision

Samples of known rat growth hormone concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	1.60	3.12	9.89
Standard Deviation	0.08	0.19	0.33
% Coefficient of Variation	5.0	6.1	3.3

Expected values

Thirty random serum and plasma samples, as well as four samples for rats at various stages of pregnancy were evaluated for the presence of rat growth hormone in this assay.

Sample	Range (ng/mL)	Average (ng/mL)
Serum (n=11)	3.86–93.3	20.35
EDTA plasma (n=7)	4.15–21	7.13
Citrate plasma (n=8)	1.69–61.6	15.38
Mid stage pregnant serum (n=3)	3.28–6.79	5.38
Late stage pregnant serum (n=1)	25.3	25.3

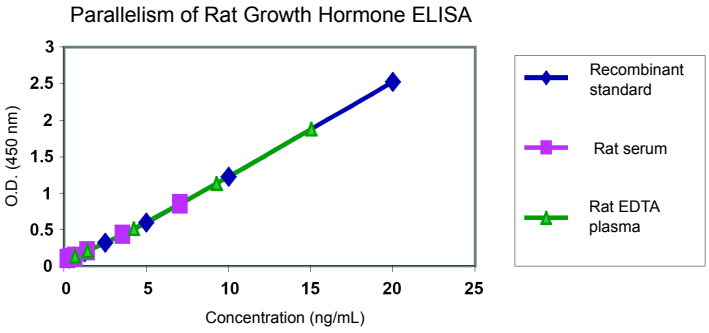
Linearity of dilution

Rat serum, EDTA plasma, and tissue culture medium containing or spiked with recombinant rat growth hormone were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded the following correlation coefficients.

Sample	Average correlation coefficient
Serum	0.99
EDTA plasma	0.98
Tissue culture media	0.99

Parallelism

Random rat serum and plasma samples were serially diluted in the Standard Diluent Buffer. The optical density of each dilution was plotted against the rat growth hormone standard curve. The standard accurately reflects the rat growth hormone content in natural samples.



Recovery

The recovery of recombinant rat growth hormone added to rat serum, plasma, or cell culture medium was measured with the Rat Growth Hormone ELISA Kit. All samples were pre-diluted 1:5 as described in the sample preparation procedure.

Sample	Average % recovery
Serum	109.8
EDTA plasma	100.7
Citrate plasma	106.3
DMEM +10% fetal bovine serum	106.9
RPMI +10% fetal bovine serum	103.6

Sensitivity

The analytical sensitivity of the assay is <0.5 ng/mL rat growth hormone. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of a panel of substances ranging in concentration from 1.32 to 60.94 ng/mL were assayed with the Rat Growth Hormone ELISA Kitand found to have no cross-reactivity: **human** eotaxin, GM-CSF, growth hormone, IL-1α, IL-1β, IL-2,IL-3, IL-4, IL-5,IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α, IFN-γ, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, RANTES, TNF-α; **mouse** FGFb, GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IP-10, IFN-γ, KC, MCP-1, MIG, MIP-1α, TNF-α, VEGF; **rat** GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, prolactin, TNF-α.

Cross-reactivity

The cross-reactivity with recombinant mouse growth hormone was determined to be 100%.

No cross-reactivity was observed with human, goat, rabbit, monkey, or swine serum samples, but significant cross-reactivity was observed with hamster and mouse serum samples.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.