User Guide

Rat/Mouse Growth Hormone ELISA

96-Well Plate

EZRMGH-45K

For research use only. Not for use in diagnostic procedures.

Intended Use

This Rat/Mouse Growth Hormone ELISA kit is used for the non-radioactive quantification of Growth Hormone in rat or mouse serum, plasma, tissue extracts or cell culture media samples. One kit is sufficient to measure 39 unknown samples in duplicate.

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Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- Capture of rat or mouse growth hormone molecules from samples to the wells of a microtiter plate coated with pre-titered amount of anti-Growth Hormone polyclonal antibody
- Washing of unbound materials from samples
- Binding of a second biotinylated anti-Growth Hormone polyclonal antibody to the captured molecules
- Washing of unbound materials from samples,
- Binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies
- Washing of excess free enzyme conjugates, and
- Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3’,5,5’-tetramethylbenzidine.

The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured rat or mouse growth hormone in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Rat Growth Hormone.

Reagents Supplied

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Volume	Quantity	Cat. No.
Microtiter Plate with 2 plate sealers **Note:** Unused strips should be resealed in the foil pouch with the desiccant and stored at 2-8 °C.	-	1 plate 2 sealers	EP45
Rat/Mouse Growth Hormone Standard	Lyophilized	1 vial	E8045-K
Rat/Mouse Growth Hormone Quality Controls 1 and 2	Lyophilized	2 vials	E6045-K
Stop Solution 0.3 M HCl (**Caution:** Corrosive Solution)	12 mL	1 vial	ET-TMB
Assay Buffer Buffer containing BSA and 0.08% Sodium Azide	40 mL	1 vial	EAB-P
10X Wash Buffer 10X concentrate of 50 mM Tris Buffered Saline containing Tween® 20	50 mL	2 bottles	EWB-HRP
Rat/Mouse Growth Hormone Detection Antibody	12 mL	1 bottle	E1045
Enzyme Solution	12 mL	1 bottle	EHRP
Substrate Solution 3,3’,5,5’-tetramethylbenzidine in buffer (Light sensitive, avoid unnecessary exposure to light)	12 mL	1 bottle	ESS-TMB

Storage and Stability

Recommended storage for kit components is 2-8 °C.

All components are shipped and stored at 2-8 °C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Symbol Definitions

Ingredient	Cat No.	Full Label	
Rat/Mouse Growth Hormone Detection Antibody	E1045		**Warning**. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Rat/Mouses Growth Hormone Quality Control 1 & 2	E6045-K		**Danger**. Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Rat/Mouses Growth Hormone Standard	E8045-K		**Danger**. Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention.
Assay Buffer	EAB-P		**Warning**. Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Stop Solution	ET-TMB		**Warning**. May be corrosive to metals.
10X HRP 10X Wash Buffer Concentrate	EWB-HRP		**Warning**. May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Reagent Precautions

Sodium Azide

Sodium azide or Proclin™ has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide and Proclin™ may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

Sample Collection and Storage

Preparation of Serum & Plasma Samples

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 minutes. Promptly centrifuge the clotted blood at 2,000 to 3,000 x *g* for 15 minutes at 4 ±2 °C. Transfer and store serum samples in separate tubes. Date and identify each sample. Use freshly prepared serum or aliquot and store samples at ≤ −20 °C for later use. For long-term storage, keep at −70 °C. Avoid freeze/thaw cycles.
2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
4. Avoid using samples with gross hemolysis or lipemia.

Sample Preparation

1. No dilution or preparation is needed for normal serum or plasma samples. In the event that any sample is above 50 ng/mL range, dilutions should be performed using the Assay Buffer provided.
2. Tissue extracts or cell culture samples may require dilution. Dilutions should be performed using the Assay Buffer provided.

Reagent Preparation

Rat/Mouse Growth Hormone Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Rat/Mouse Growth Hormone Standard with 0.5 mL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label six tubes 16.7, 5.6, 1.9, 0.62, 0.21, and 0.07 ng/mL. Add 100 µL Assay Buffer to each of the six tubes. Prepare 3 times serial dilutions by adding 50 µL of the 50 ng/mL reconstituted standard to the 16.7 ng/mL tube, mix well and transfer 50 µL of the 16.7 ng/mL standard to the 5.6 ng/mL tube, mix well and transfer 50 µL of the 5.6 ng/mL standard to the 1.9 ng/mL tube, mix well and transfer 50 µL of the 1.9 ng/mL standard to the 0.62 ng/mL tube, mix well and transfer 50 µL of the 0.62 ng/mL standard to the 0.21 ng/mL tube, mix well and transfer 50 µL of the 0.21 ng/mL standard to the 0.07 ng/mL tube and mix well.

   Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Standard Concentration (ng/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
50	0.5 mL	0

Standard Concentration (ng/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
16.7	100 µL	50 µL of 50 ng/mL
5.6	100 µL	50 µL of 16.7 ng/mL
1.9	100 µL	50 µL of 5.6 ng/mL
0.62	100 µL	50 µL of 1.9 ng/mL
0.21	100 µL	50 µL of 0.62 ng/mL
0.07	100 µL	50 µL of 0.21 ng/mL

Rat/Mouse Growth Hormone Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each Rat/Mouse Growth Hormone Quality Control 1 and Quality Control 2 with 0.5 mL distilled or de-ionized water and invert and mix gently, let sit for 5 minutes then mix well to ensure complete hydration.

Assay Procedure

Warm all reagents to room temperature before setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10-fold by mixing the entire contents of both buffer bottles with 900 mL deionized or distilled water.
2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8 °C. Assemble the strips in an empty plate holder. Add 300 µL diluted Wash Buffer to each well of the plate. Incubate at room temperature for 5 minutes. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol following the manufacturer's instructions for all washing steps described in this protocol.
3. Add in duplicate, 100 µL Assay Buffer to each of the Blank wells.
4. Add in duplicate, 90 µL Assay Buffer to Standard wells, QC1, QC2, and sample wells (see Microtiter Plate Arrangement for suggested well orientation).
5. Add in duplicate, 10 µL Rat/Mouse Growth Hormone Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 10 µL QC1 and 10 µL QC2 to the appropriate wells. Add sequentially, 10 µL of the unknown samples in duplicate to the remaining wells. For best result all additions should be completed within 30 minutes.
6. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
8. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
9. Add 100 µL Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
11. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
12. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.
13. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
14. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

15. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5 to 20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of Growth Hormone standards with intensity proportional to increasing concentrations of Growth Hormone.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time. One can monitor color development using a 370 nm filter, if available, on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.

16. Remove the sealer and add 100 µL Stop Solution (**Caution:** Corrosive Solution) and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest Growth Hormone standard should be approximately 2.0-3.2, or not to exceed the capability of the plate reader used.

Assay Procedure for Rat/Mouse Growth Hormone ELISA Kit

	Step 1	Step 2	Step 3-4	Step 5	Step 6-8	Step 9	Step 9-11	Step 12	Step 12-14	Step 15		Step 16	
Well #	Dilute both bottles of 10X Wash Buffer with 900 mL Deionized Water.	Wash plate with 300 µL Wash Buffer and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels.	Assay Buffer	Standards/ Controls/ Samples	Seal, agitate, incubate 1.5 hours at room temperature. Wash 3X with 300 µL Wash Buffer.	Detection Ab	Seal, agitate, incubate 1hour at room temperature. Remove residual buffer by tapping smartly on absorbent towels Wash 3X with 300 µL Wash Buffer.	Enzyme Solution	Seal, agitate, incubate 30 minutes at room temperature. Wash 3X with 300 µL Wash Buffer.	Substrate	Seal, agitate, incubate 5-20 minutes at room temperature.	Stop Solution	Read absorbance at 450 nm and 590 nm.
A1 B1			100 µL	-		100 µL		100 µL		100 µL		100 µL	
C1 D1			90 µL	10 µL of 0.07 ng/mL Std.									
E1 F1			90 µL	10 µL of 0.21 ng/mL Std.									
G1 H1			90 µL	10 µL of 0.62 ng/mL Std.									
A2 B2			90 µL	10 µL of 1.9 ng/mL Std.									
C2 D2			90 µL	10 µL of 5.6 ng/mL Std.									
E2 F2			90 µL	10 µL of 16.7 ng/mL Std.									
G2 H2			90 µL	10 µL of 50 ng/mL Std.									
A3 B3			90 µL	10 µL of QC 1									
C3 D3			90 µL	10 µL of QC 2									
E3 F3			90 µL	10 µL of Sample									
G3 H3			90 µL	10 µL of Sample									

Microtiter Plate Arrangement

Rat/Mouse Growth Hormone ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1.9 ng/mL	QC 1	Sample 3								
B	Blank	1.9 ng/mL	QC 1	Sample 3								
C	0.07 ng/mL	5.6 ng/mL	QC 2	Etc								
D	0.07 ng/mL	5.6 ng/mL	QC 2									
E	0.21 ng/mL	16.7 ng/mL	Sample 1									
F	0.21 ng/mL	16.7 ng/mL	Sample 1									
G	0.62 ng/mL	50 ng/mL	Sample 2									
H	0.62 ng/mL	50 ng/mL	Sample 2									

Calculations

The dose-response curve of this assay fits best to a 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

Note: When sample volumes assayed differ from 10 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (For example: if 5 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µL, compensate the volume deficit with Assay Buffer.

Interpretation

Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.

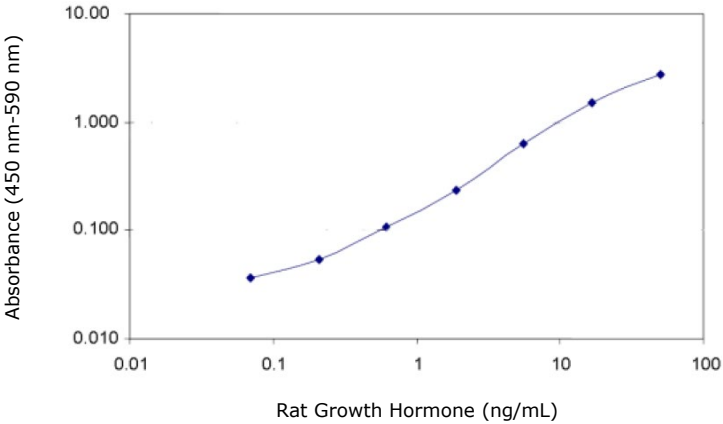
2. If the difference between duplicate results of a sample is > 10% CV, repeat the sample.

3. The limit of sensitivity of this assay is 0.07 ng/mL Rat/Mouse Growth Hormone (10 µL sample size).

4. The appropriate range of this assay is 0.07 ng/mL to 50 ng/mL Rat/Mouse Growth Hormone (10 µL sample size). Any result greater than 50 ng/mL in a 10 µL sample should be diluted using Assay Buffer, and the assay repeated until the results fall within range. Tissue extracts or cell culture media samples greater than 50 ng/mL in a 10 µL sample should be diluted in Assay Buffer.

Graph of Typical Reference Curve

Rat/Mouse Growth Hormone ELISA



Typical Standard Curve, not to be used to calculate data.

Assay Characteristics

Sensitivity

The lowest level of Rat/Mouse Growth Hormone that can be detected by assay is 0.07 ng/mL when using a 10 µL sample size.

Specificity

Rat Growth Hormone	100 %
Rat ACTH	n.d.
Rat TSH	n.d
Rat FSH	n.d
Rat LH	n.d
Rat Prolactin	n.d
Rat BDNF	n.d
Human Growth Hormone	n.d

n.d. = Not Detectable May cross react with hamster, canine, and primate growth hormone.

Precision

Intra-Assay Variation

	Mean GH Levels (ng/mL)	Intra-Assay %CV
1	4.1	2.3
2	2.7	4.3
3	2.6	2.1
4	6.1	1.7

Inter-Assay Variation

	Mean GH Levels (ng/mL)	Inter-Assay % CV
1	11	4.5
2	5.6	3.2
3	5.2	4.9

The assay variations of Rat/Mouse Growth Hormone ELISA kits were studied on four rat serum samples with varying concentrations of endogenous Growth Hormone. The mean intra-assay variation was calculated from results of six duplicate determinations in each assay of the indicated samples. The mean inter-assay variation of each sample was calculated from results of four separate assays with duplicate samples in each assay.

Spike & Recovery of Rat Growth Hormone in Rat Serum Samples

Sample	GH Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery%
1	0	1.74	1.74	100
	3.125	4.865	4.79	98
	6.25	7.99	7.82	98
	12.5	14.24	13.91	98
2	0	3.13	3.13	100
	3.125	6.255	6.13	98
	6.25	9.38	9.15	98
	12.5	15.63	15.78	101
3	0	2.42	2.42	100
	3.125	5.545	5.35	96
	6.25	8.67	8.39	97
	12.5	14.92	14.92	100

Varying amounts of Rat Growth Hormone were added to three rat serum samples and the Growth Hormone content was determined. The % of recovery = observed Growth Hormone concentrations/expected Growth Hormone concentrations x 100%.

Linearity

Effect of Serum Dilution

Sample	Volume (µL)	Expected (ng/mL)	Observed (ng/mL)	Expected%
1	10	20	20	100
	7.5	15	15.5	103
	5	10	11.2	112
	2.5	5	5.9	118
2	10	4.8	4.8	100
	7.5	3.6	3.6	100
	5	2.4	2.5	104
	2.5	1.2	1.3	108
3	10	3.9	3.9	100
	7.5	2.9	2.8	97
	5	1.95	1.97	101
	2.5	.98	1.0	102

Three rat serum samples with the indicated sample volumes were assayed. Required amounts of matrix were added to compensate for lost volumes below 10 µL. The resulting dilution factors of 1.0, 1.3, 2.0, and 4.0 representing 10 µL, 7.5 µL, 5 µL, and 2.5 µL sample volumes assayed, respectively, were applied in the calculation of observed Growth Hormone concentrations. % of expected = observed/expected x 100%.

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- Do not let the absorbency reading of the highest standard reach 3.2 units or higher after acidification.
- High absorbance in background or blank wells could be due to:
    - cross well contamination by standard solution or sample
    - inadequate washing of wells with Wash Buffer
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
Rat/Mouse Growth Hormone ELISA Plate	EP45
10X HRP Wash Buffer Concentrate	EWB-HRP
Rat/Mouse Growth Hormone ELISA Standard	E8045-K
Rat/Mouse Growth Hormone Quality Controls 1 and 2	E6045-K
Stop Solution	ET-TMB
Assay Buffer	EAB-P
Rat/Mouse Growth Hormone ELISA Detection Antibody	E1045
Enzyme Solution	EHRP
Substrate Solution	ESS-TMB

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