Catalog #80587

V.5

Crystal Chem
High Performance Assays

Mouse Growth Hormone ELISA Kit Instructions

For the quantitative determination of growth hormone in mouse serum

Catalog #80587
96 Assays

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

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

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A. Intended Use

The Mouse Growth Hormone ELISA kit is for the quantitative determination of growth hormone in mouse serum. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Growth hormone is a peptide hormone that stimulates growth, cell reproduction and regeneration in mice, humans, and other animals.

C. Principle of the Assay

The Mouse Growth Hormone ELISA kit is a sandwich ELISA assay for mouse growth hormone. In the assay, the mouse growth hormone present in the sample binds with anti-growth hormone antibodies adsorbed to the surface of a microplate. In the following step, a biotinylated antibody is added and binds to the immobilized growth hormone. After washing, a HRP-streptavidin conjugate is added to form a complex with the biotinylated antibody bound on the surface. In the closing substrate reaction, an enzyme substrate is added, and the growth hormone levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Mouse Growth Hormone ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-7	Standards	1 x 7 vials
DIL	Dilution Buffer	1 x 50 mL
CON1-2	Controls	1 x 2 vials
AB CONJ	Antibody Conjugate	1 x 12 mL
ENZ CONJ	Enzyme Conjugate	1 x 12 mL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Sealing tape for plate	3x, adhesive

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks

Vortex mixer
Microplate shaker (350 rpm)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 100 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Mouse serum samples can be used. Samples should be chilled as soon as possible after sample withdrawal. For long-term storage, samples can be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Hemolytic samples should be avoided.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-7
   Standards are provided in lyophilized form with concentrations ranging from 0.15 ng/mL to 9.0 ng/mL. Dilute each standard with 1 mL of Dilution Buffer (marked "DIL"). After reconstitution, it is recommended that standards be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted standards are stable for four weeks at -20°C. Standards should be not be repeatedly thawed (at most, once), so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. Standards are provided in the following concentrations: 0.15, 0.45, 0.9, 1.8, 3.6, 6.0 and 9.0 ng/mL.
3. Dilution Buffer
   Provided as ready to use.
4. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute controls with 150 µL of Dilution Buffer.

5. Antibody Conjugate
   Provided as ready to use.
6. Enzyme Conjugate
   Provided as ready to use.
7. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for 4 weeks at 2-8°C after dilution, so dilute only as needed.
8. Substrate Solution
   Provided as ready to use.
9. Stop Solution
   Provided as ready to use.

I.2. Dilution of samples and controls

1. Samples and controls need to be diluted with Dilution Buffer for use with the assay. Please note that this section only applies to samples and controls, not standards. A sample dilution of 1:5 is recommended. A1:5 sample dilution can be performed as follows:

   For double determination, dilute 1:5 by mixing 50 µL of sample or control with 200 µL of Dilution Buffer.

   Since mouse growth hormone levels can vary, dilution ratio may need to be adjusted as appropriate. Samples and controls must be used within 60 minutes once diluted.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or 100 µL of standard or 100 µL of diluted control and mix well by repeated pipetting.
   Note: *A blank using 100 µL of Dilution Buffer is recommended.*
2. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
3. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of the Antibody Conjugate in each well.
5. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
6. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Enzyme Conjugate in each well.
8. Cover the wells with sealing tape and incubate the plate for 30 minutes at room temperature (shake at 350 rpm).
9. Aspirate well contents and wash five times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
10. Add 100 µL of Substrate Solution in each well.

11. Incubate the plate for 30 mins in dark room at room temperature.
12. Stop the reaction by adding 100 µL of Stop Solution.
13. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the growth hormone concentration

1. Using computer software, construct the growth hormone calibration curve by plotting the blank corrected mean absorbance value for each standard on the Y axis versus the corresponding mouse growth hormone concentration on the X axis. Blank corrected values are determined by subtracting the mean absorbance value of the blank from the mean absorbance value for each standard. A four parametric logistic (4-PL) curve fit, a higher-grade polynomial curve fit, or non-linear regression are suitable for the evaluation.
2. Mouse growth hormone concentrations in the samples are interpolated using the calibration curve and blank corrected mean absorbance values for each sample. For diluted samples and controls, the values obtained must be multiplied by the dilution factor (ie 5) to obtain the final growth hormone concentration. The growth hormone concentration is expressed in ng/mL.
   Note: *Samples with high mouse growth concentrations (ie. fall above the range of the assay) should be further diluted with the dilution buffer and rerun.*

J. Performance characteristics

J.1. Assay range

The Mouse Growth Hormone ELISA Kit has an assay range from 0.15 – 9.0 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

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