MEDNA™

96-Well Human Growth Hormone (HGH) ELISA Kit

Catalog number: D1003

For the quantitative determination of human growth hormone (HGH) in cell culture supernatant and serum

* This assay is specific for hGH, and does not cross react with hCG, TSH, LH and prolactin.

* This package insert must be read in its entirety before using this product.

Revision: 12/2020

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTRODUCTION

Human growth hormone (hGH, somatotropin) is a polypeptide secreted by the anterior pituitary. It is 191 amino acids in length and has a molecular mass of approximately 22,000 daltons. hGH measurement is primarily of interest in the diagnosis and treatment of various forms of abnormal growth hormone secretion. The Human Growth Hormone ELISA Kit provides a rapid, sensitive and reliable test. There is no cross-reactivity with hCG, TSH, LH and prolactin.

PRINCIPLE OF THE ASSAY

The hGH ELISA Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal anti-HGH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-HGH antibody in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in HGH molecules being sandwiched between the solid phase and enzyme-linked antibodies. Following an incubation and a wash to remove unbound labeled antibodies, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in proportion to the amount of HGH bound in the initial step. The sample values are then read off the standard curve.

PRECAUTION

The Stop Solution provided in this kit is a solution of low pH. Wear eye, hand, face and clothing protection when using this material.

MATERIALS PROVIDED

	Description	Parts
1	**Pre-coated Microplate** - 96 well polystyrene microplates coated with antibody specific for human growth hormone	2 plates
2	**HGH Standard** - 300 ng/mL	2 vials (0.8 mL)
3	**HGH Detection Antibody (Ready to use)**	1 vial (12 mL)
4	**Sample Diluent -** 1x	1 bottle (60 mL)
5	**Wash Buffer -** 20x Concentrate	1 bottle (60 mL)
6	**Color Reagent -** TMB substrate	1 bottle (12 mL)
7	**Stop Solution**	1 bottle (12 mL)
8	**Product Insert** - product description, assay protocol, material data sheet	1 booklet

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, optionally with correction wavelength set at 540 or 570 nm
- Pipettes and pipette tips
- Polypropylene test tubes
- Deionized or distilled water

STORAGE

- Do not freeze
- Store at 2-8ºC
- Best used within 3 month of receipt

SAMPLE PREPARATION

Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20ºC. Avoid repeated freeze-thaw cycles.

Serum - Allow samples to clot for 2 hours at room temperature or overnight at 2-8ºC before centrifuging for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or store samples at ≤ -20ºC. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20ºC. Avoid repeated freeze-thaw cycles.

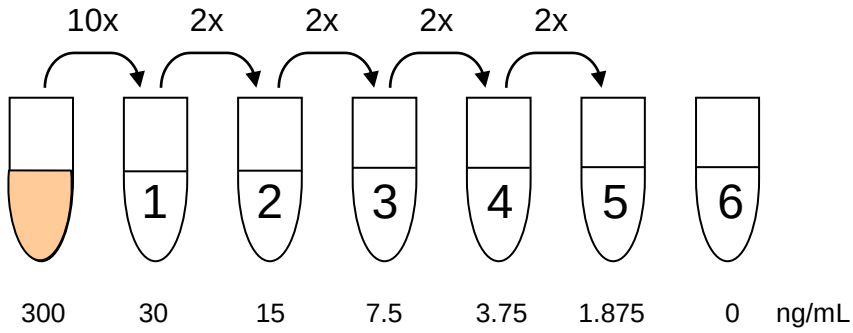
REAGENT PREPARATION

1. All reagent should be brought to room temperature (18-22ºC) before use.

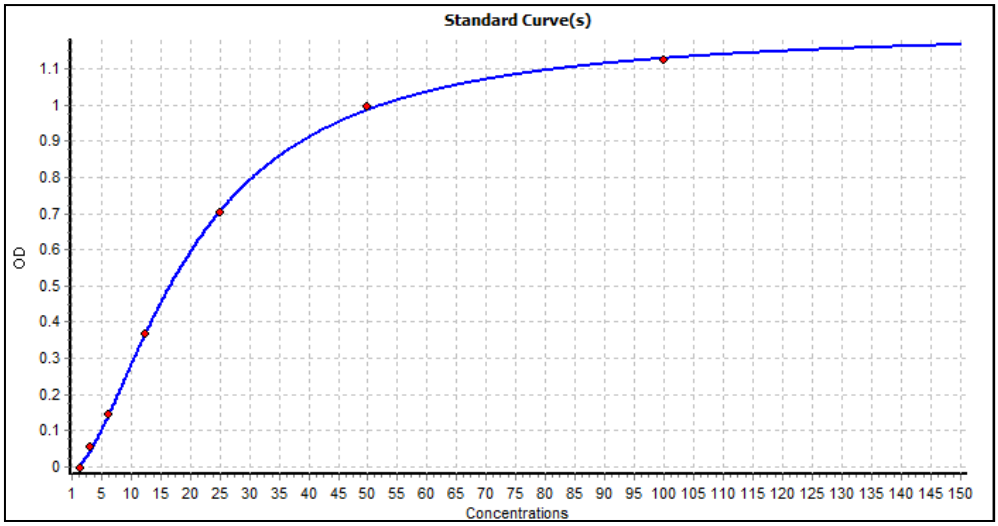
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer Concentrate (20x) into distilled water to prepare 300 ml of washing buffer (1x). Mix well before use.

3. Preparation of standard: Appropriate standard preparation will be determined during the development process.
   The series dilution procedure as followings: Add 1.8ml Sample Diluent into the first tube. Then, pipette 1ml Sampled Diluent into the following each tube. Gently vortex 300ng/ml standard stock, then add 0.2ml of 300ng/ml standard stock into the first tube to prepare a 30 ng/ml standard 1. Mix the first tube well, draw 1ml standard 1 solution from the first tube into the second tube to make a 15 ng/ml standard 2. Use the same method to make the following standards. Gently Vortex each tube thoroughly before the next transfer.

Recommended HGH standard serial dilution:



Sample HGH standard curve (4-PL fit)



HGH Standard (ng/mL)

Note on standard curve linearity: The linearity of standard curves is influenced by various factors including incubation time, temperature, amount of secondary antibody, and reaction substrate. With advanced data analysis software, standard curves do not need to be perfectly linear for suitable data analysis and conversion. Medna uses and recommends BMG LABTECH Microplate Readers and MARS Data Analysis Software (www.bmglabtech.com).

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standard, specimens, and controls into appropriate wells.
3. Dispense 50µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this setup.
5. Incubate at room temperature (18-22ºC) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 50µl of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature in the dark for 20 minutes.
11. Stop the reaction by adding 50µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter reader within 30 minutes.

Important Note:

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances readings.

SENSITIVITY

The minimal sensitivity of the test is 0.5 ng/ml.

MATERIAL SAFETY DATA
Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)

Hazards Identification:
STOP SOLUTION (0.1 N hydrochloric acid) is a diluted hydrochloric acid. Hydrochloric acid is a hazardous material. CAS NO.7647-01-0. Molecular Weight: 36.46 Chemical Formula: HCl

Emergency Overview
POISON! DANGER! CORROSIVE. LIQUID AND MIST CAUSE SEVERE BURNS TO ALL BODY TISSUE. MAY BE FATAL IF SWALLOWED OR INHALED. INHALATION MAY CAUSE LUNG DAMAGE.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 3 - Severe (Poison)
Flammability Rating: 0 - None
Reactivity Rating: 2 - Moderate
Contact Rating: 4 - Extreme (Corrosive)
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES
Storage Color Code: White (Corrosive)

Personal Protection
Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, and gloves. Avoid contact of material with skin or eyes. Ensure access to a safety shower and eye-wash.

First Aid Measures
Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Ingestion: DO NOT INDUCE VOMITING! Give large quantities of water or milk if available. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Skin Contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.
Eye Contact: Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.