Human HDGF ELISA Kit

Enzyme Immunoassay kit for the quantification of Human HDGF in serum, plasma and cell culture supernatant samples.

Catalog number: ARG81356

Package: 96 wells

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INTRODUCTION

HDGF is a member of the hepatoma-derived growth factor family. The encoded protein has mitogenic and DNA-binding activity and may play a role in cellular proliferation and differentiation. This gene was thought initially to be located on chromosome X, however, that location has been determined to correspond to a related pseudogene. Alternatively spliced transcript variants encoding distinct isoforms have been described. [provided by RefSeq, Jul 2008]

HDGF is a Heparin-binding protein, with mitogenic activity for fibroblasts. Acts as a transcriptional repressor. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of Human HDGF in serum, plasma and cell culture supernatant samples. An antibody specific for HDGF has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any HDGF present is bound on the plate. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated primary antibody binds to HDGF is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of total HDGF bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of total HDGF in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (50 ng)	1 vial	4°C, store at -20 °C after reconstitution
Standard reconstitution buffer	1.1 ml (ready-to-use)	4°C
HRP-antibody Conjugate (100X)	115 µl	4°C
Sample/Antibody diluent buffer	30 ml (ready-to-use)	4°C
Assay Buffer	6 ml (ready-to-use)	4°C
10X Wash buffer	22 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)
STOP solution	6 ml (ready-to-use)	4°C
Plate sealer	1 strip	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved. 1X Wash buffer should be prepared and stored at 4°C before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. It is important to

ensure a platelet free preparation since platelets can release HMGB1. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles. Avoid using haemolytic, icteric or lipaemic samples and samples contain azide cannot be assayed.

Cell Culture Supernatants: It is recommended to use serum-free medium or use BSA to replace serum in cell culture medium or use chemical modified serum free medium for cell culture. Users should choose suitable culture medium based on the cell types. Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Standard**: Reconstitute the standard with **1.0 ml Standard reconstitution buffer** to yield a stock concentration of **50 ng/ml**. Mix the standard by inversion or brief vortex for 5-10 seconds (do not vortex at high-speed) and stand for 10 minutes at RT to completely dissolve contents. Aliquot and store at ≤ -20°C for up to 2 weeks (store at -80°C is recommended, freeze-thaw the reconstituted standard for only once).

  Make sure the standard is dissolved completely before making serial dilutions. The **Sample/Antibody diluent buffer** serves as zero standard (0 pg/ml), and the standard stock can be diluted with Sample/Antibody diluent buffer as according to the suggested concentration below: **5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml and 78.125 pg/ml**.

Dilute HDGF standard as according to the table below:

Standard	HDGF Conc. (pg/ml)	µl of Sample/Antibody diluent buffer	µl of standard
S7	5000	450	50 (50 ng/ml Stock)
S6	2500	250	250 (S7)
S5	1250	250	250 (S6)
S4	625	250	250 (S5)
S3	312.5	250	250 (S4)
S2	156.25	250	250 (S3)
S1	78.125	250	250 (S2)
S0	0	250	0

- **1X Wash buffer**: Dilute 10X wash buffer with distilled water to yield 1X wash buffer. The diluted Wash buffer should be stored at 4 °C.
- **HRP-antibody Conjugate:** Diluent 100X HRP-antibody Conjugate with Sample/Antibody diluent buffer to yield 1X HRP-antibody Conjugate solution. The 1X HRP-antibody Conjugate solution should be used immediately.
- **Sample:**

  - If the sample volume is less than 50 µl, add Sample/Antibody diluent buffer to a final volume of 50 µl, for the calculation of the concentrations this dilution factor has to be taken into account.

  - If the initial assay found samples contain HDGF higher than the highest standard, the samples can be diluted with Sample/Antibody diluent buffer and re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

Dilution 1:10, 20 µl sample + 180 µl Sample/Antibody diluent buffer (mix thoroughly).

ASSAY PROCEDURE

All materials do not need equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards, samples and zero controls** into appropriate wells.
3. Add **50 µl** of **Assay buffer** into **all wells** immediately. Mix thoroughly by gently shaking or tapping the plate. Cover the plate and **incubate for 2 hour at room temperature.**
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **1X HRP-antibody conjugate** into each well. Mix thoroughly by gently shaking or tapping the plate. Cover wells and incubate for **1 hour at room temperature** in dark.
6. Aspirate each well and **wash as step 4**.
7. Add **100 µl** of **TMB substrate** to each well. Incubate for **10 minutes at RT** in dark. Substrate will change from colorless to different strengths of blue.
8. Add **50 µl** of **Stop solution** to each well. The color of the solution should

change from blue to yellow. Mix thoroughly by gently shaking the plate.

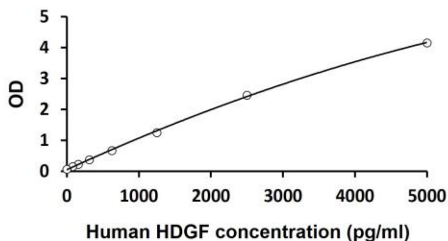
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Standard range: 78.125 – 5000 pg/ml

Minimum Detectable Concentration: 30 pg/ml

Precision:

The CV values of both intra and inter precision fall below 10%.

Recovery:

94-102 %

Specificity

This assay recognizes natural and recombinant human HDGF protein.