HUMAN HEPATOCYTE GROWTH FACTOR ACTIVATOR (HGFA) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN HGF ACTIVATOR CONCENTRATIONS IN SERUM AND PLASMA.

PURCHASE INFORMATION:

ELISA NAME	HUMAN HGFA ELISA
Catalog No.	SK00331-08
Formulation	96 T
Standard Range	22-1400 pg/ml
Sensitivity	10 pg/mL
Sample Volume	100 µl per well
Sample Type	Serum, plasma
Specificity	Human HGFA
Sample Dilution	
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

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FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

This Human HGF Activator (HGFA) ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human HGFA from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human HGFA and antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human HGFA samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human HGFA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human HGFA present is bound by the immobilized antibody. After washing away any unbound substances, antibody HRP conjugate specific for human HGFA is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human HGFA bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Human HGFA Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human HGFA	331-08-01	1 plate
Human HGFA Standard – 1.4 ng/vial of recombinant human HGFA in a buffered protein base with preservatives; lyophilized.	331-08-02	1 vial
Detection Antibody HRP Conjugate – 110 µL / vial, 100-fold concentrated of Antibody HRP conjugate against human HGFA with preservatives;	331-08-03	1 vial
Positive Control- one of human HGFA, lyophilized	331-08-04	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB10	1 vial
HRP Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	DB06	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution -11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: Reconstituted Detection Antibody and Standard Stock may be stored for up to 1 month at -70°C. Streptavidin HRP conjugate 100 fold concentrated should be stored at 2 - 8° C .
Microplate Wells: Return unused wells to the plastic zip bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

Human HGFA Standard – Reconstitute the human HGFA standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 1400 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	1400 pg/ml
# 1	250 µl of stock	250 µl	700 pg/ml
# 2	250 µl of 1	250 µl	350 pg/ml
# 3	250 µl of 2	250 µl	175 pg/ml
# 4	250 µl of 3	250 µl	87.5 pg/ml
# 5	250 µl of 4	250 µl	43.75 pg/ml
# 6	250 µl of 5	250 µl	21.875 pg/ml

Positive Control - Reconstitute the Positive Control with 1 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20° C or -70° C.

Detection Antibody-HRP Conjugate - Pipette 10.89 mL of **Detection Antibody-HRP Diluent Solution (DB10)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection IgG-HRP should be used within a few days **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution from #6-S (B2, B3 to H2, H3), **sample,** or **positive control** (H4, H5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody-HRP Conjugate working solution**. Cover with plate sealer and incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of **Substrate Solution**. Incubate for 10-15 minutes on microplate shaker at room temperature. **Protect from light.**
9. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Read plate using a microplate reader set to 450 nm within 15 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

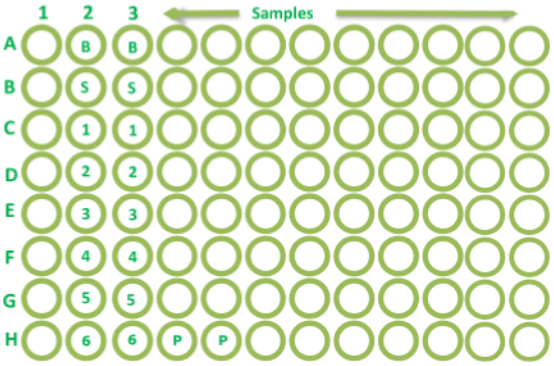
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human HGFA.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human HGFA was 10 pg/mL.



TYPICAL DATA

These standard curves* are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.093)
21.875	0.055
43.75	0.108
87.5	0.211
175	0.451
350	0.907
700	1.743
1400	2.817

SPECIFICITY

PROTEINS	CROSS-REACTIVITY(%)
Human HGFA	100
Human HGF	0
Human VDBP	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate 1 hour on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 10-15 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 15 minutes.