HUMAN HEPATOCYTE GROWTH FACTOR ALPHA CHAIN (HGF-α) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN HGF-ALPHA CONCENTRATIONS IN THE RECOMBINANT PROTEIN SAMPLES

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN HGF-ALPHA ELISA KIT**
Catalog No.	SK00331-01
Lot No.	20112311
Formulation	96 T
Standard range	3.125 ~ 200 ng/mL
Sensitivity	1000 pg/mL
Sample volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Recombinant Protein
Specificity	Human HGF-α
Calibration	Human HGF-α recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months. Check page 2 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

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DESCRIPTION

This Human HGF-alpha ELISA Kit contains the necessary components required for the quantitative measurement of recombinant human HFG-alpha in a sandwich ELISA format.

This immunoassay contains recombinant human HFG-alpha and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant HGF-alpha samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human HGF-alpha . The capture antibody can bind to the human HGF-A in the standard and samples. After washing the plate of any unbound substances, a polyclonal antibody against human HGF-A is added to the wells. After another washing of the plate, Anti Rabbit IgG-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human HGF-A bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
HGF-A Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against HGF-A .	**331-01-01**	**1 plate**
HGF-A Standard – 200 ng /vial of recombinant human HGF-A in a buffered protein base with preservative; lyophilized.	**331-01-02**	**1 vial**
Detection Antibody – 1.2 mL/vial, 10-fold concentrate of a purified polyclonal antibody against HGF-A with preservative; lyophilized.	**331-01-03**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Goat Anti Rabbit IgG conjugate to HRP.	**ARIGHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB11C**	**1 bottle**
Wash Buffer 20X - 25mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For longer storage for up to 8 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Anti Rabbit IgG-HRP Conjugate should be stored only at 2 -8 °C. Do not use kit past expiration date.

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 SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

HGF-alpha Standard - Reconstitute the HGF-alpha standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 200ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #2 to #6. Use the 200 ng/mL solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **200 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	200 ng/ml
# 1	150µl of stock	450µl	100 ng/ml
# 2	250µl of 1	250µl	50 ng/ml
# 3	250µl of 2	250µl	25 ng/ml
# 4	250µl of 3	250µl	12.5 ng/ml
# 5	250µl of 4	250µl	6.25 ng/ml
# 6	250µl of 5	250µl	3.125 ng/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Dilution Buffer DB11C** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Dilution Buffer DB11C** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Anti-Rabbit IgG-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Anti-Rabbit IgG-HRP conjugate stock solution to 11.88 mL **Dilution Buffer DB11C** to prepare working solution *(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 and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions from #6 to #S, samples, per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four 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 step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

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.

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human HGF-alpha	100%
Human HGF-beta	0

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.119)
3.125	0.036
6.25	0.079
12.5	0.159
25	0.318
50	0.627
100	1.109
200	2.209

- **Lot No.: 20112311**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Anti Rabbit IgG HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate 15-20 min on the plate shaker at RT. **Protect from light.**

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Add 100 µl Stop Solution to each well. Read 450nm within 5 min.