

HIGH SENSITIVITY HGF- ALPHA HUMAN ELISA KIT

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



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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	High Sensitivity HGF-Alpha Human ELISA Kit
Catalog No.	SK00331-06
Lot No.	20114631
Formulation	192 T (2 plates)
Standard range	0.195 ~12.5 ng/mL
Sensitivity	75 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-alpha
Calibration	Human HGF-alpha Rec
Intra-assay Precision	4 - 7%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months. See page 2-3 for detail
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human High Sensitivity HGF-alpha ELISA Kit contains the necessary components required for the quantitative measurement of recombinant HGF-alpha in a sandwich ELISA format. Other sample types need to be validated with this assay.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human HGF-alpha. The capture antibody can bind to the HGF-alpha in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human HGF-alpha is added to the wells. After another washing of the plate, a Streptavidin-HRP conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of HGF-alpha 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 with a pretest.

_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
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HGF-alpha Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human HGF-alpha.	331-06-01	1 plate
HGF-alpha Standard – 25 ng/vial of recombinant human HGF-alpha in a buffered protein base with preservative; lyophilized.	331-06-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of a purified biotinylated antibody against human HGF-alpha with preservative; lyophilized.	331-06-03	1 vial
Positive Control - one vial of recombinant human HGF-alpha; lyophilized.	331-06-04	1 vial
Streptavidin-HRP Conjugate - 100 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer - 45 mL of buffered protein based solution with preservative.	DB11C	1 bottle
Wash Buffer 20X - 25 mL 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 solution.	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

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 and Dilution Buffer (DB11C) should be stored at -20° C or -70° C. **Streptavidin-HRP Conjugate** and TMB Substrate Solution 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 PREPARATION

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

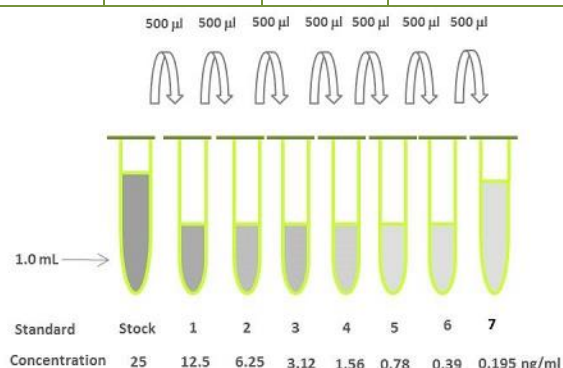
REAGENT PREPARATION

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 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

HGF-alpha Standard - Reconstitute the human HGF-alpha standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 25 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 μ L of Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **12.5 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL). Store the stock solution at -70°C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	25 ng/ml
# 1	500 μ l of stock	500 μ l	12.5 ng/ml
# 2	500 μ l of 1	500 μ l	6.25 ng/ml
# 3	500 μ l of 2	500 μ l	3.125 ng/ml
# 4	500 μ l of 3	500 μ l	1.56 ng/ml
# 5	500 μ l of 4	500 μ l	0.78 ng/ml
# 6	500 μ l of 5	500 μ l	0.39 ng/ml
# 7	500 μ l of 6	500 μ l	0.195 ng/ml



Positive Control - Reconstitute the Positive Control with 1ml of Dilution Buffer. Discard the positive control after use. This is for one time use only.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. For 96 wells test, Freshly Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. Store the stock solution at -20 °C for a few days.

Streptavidin-HRP Conjugate - For the 96 wells test, Freshly Pipette 10.89 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 110 μ L of 100-fold concentrated stock solution to prepare working solution (**protect from light**). *The working solution of Streptavidin-HRP Conjugate should be used within 20 min.* For the partial strip test, freshly prepare 900 μ L per strip of working solution. Always store the stock solution of Streptavidin-HRP Conjugate 100-fold concentrated at 2 ~ 8 °C for 8 months.

ELISA PROTOCOL

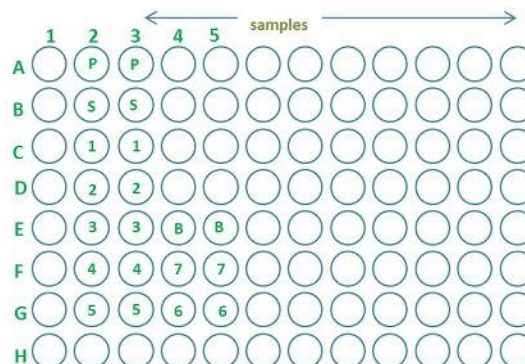
Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all samples, reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells (B).
3. Add 100 µL of standard dilutions in reverse order of serial dilution from #7 to #1, samples, or positive control (P) per well. Cover with the 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 the 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 *Streptavidin-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 using a microplate reader set to 450 nm within 3 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 or 4-Parameter 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.



TYPICAL STANDARD CURVE

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 OD450NM (CORRECTED)
Blank	0 (0.117)
0.195	0.042
0.39	0.081
0.78	0.161
1.56	0.316
3.125	0.532
6.25	1.021
12.5	1.999
25 (optional)	2.963









Lot: 20114631

Positive Control: 0.5 ~1.5 ng/mL (log-log)

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human HGF-Alpha	100%
Human HGF-Beta	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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

Aspirate and wash 4 times.

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

Aspirate and wash 4 times.

Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. Protect from light.

Aspirate and wash 4 times.

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

Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.