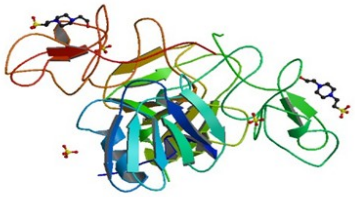
Human HGF Activator, HGFAC ELISA Kit

Prod. No.: DEIA253
Pkg.Size: 5 plates



PDB rendering based on 1bht.

INTENDED USE

The human HGFAC ELISA kit is for the quantitative determination of human HGFAC.
This ELISA kit contains the basic components required for the development of sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on five 96-well plates.

PRINCIPLE OF THE TEST

The ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for HGFAC coated on a 96-well plate. Standards and samples are added to the wells, and any HGFAC present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-HGFAC monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of HGFAC present in the sample streptavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450nm.

SPECIFICITY&SENSITIVITY

The minimum detectable dose of human HGFAC was determined to be approximately 39.1 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.
Capture Antibody: 0.5 mg/ml of mouse anti-HGFAC monoclonal antibody. Dilute to a working concentration of 2 μg/ml in CBS before coating.
Detection Antibody: Each vial contains 144μg biotinylated rabbit anti-HGFAC polyclonal antibody. Reconstitute with sterile 1ml detection antibody dilution buffer. After reconstitution, store at -20 to -70℃in a manual defrost freezer. Dilute to a working concentration of 1 μg/ml in detection antibody dilution buffer before use.
Standard: Each vial contains 100 ng of recombinant HGFAC. Reconstitute standard power with detection antibody dilution buffer. After reconstitution, store at -20 to -70℃in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 2.5 ng/ml is recommended.

ANALYTE GENE INFORMATION

Gene Name: HGFAC HGF activator [*Homo sapiens*]
Official Symbol: HGFAC
Synonyms: HGFAC; HGF activator; HGFA; MGC138395; MGC138397; EC 3.4.21.; Hepatocyte growth factor activator; Hepatocyte growth factor activator short chain; Hepatocyte growth factor activator long chain
GeneID: 3083
mRNA Refseq: NM_001528
Protein Refseq: NP_001519
MIM: 604552
UniProt ID: Q04756
Chromosome Location: 4p16
Function: peptidase activity; protein binding; serine-type endopeptidase activity

SOLUTIONS REQUIRED

CBS: 0.05M Na_2CO_3, 0.05M $NaHCO_3$, pH9.6, 0.2μm filtered
TBS: 25mM Tris, adjust pH to 7.4 by HCl
Wash Buffer: 0.05% Tween20 in TBS, pH 7.2-7.4
Blocking Buffer: 2% BSA in Wash Buffer
Sample dilution buffer: 0.1% BSA in wash buffer, pH7.2-7.4, 0.2μm filtered
Detection antibody dilution buffer: 0.5% BSA in wash buffer, pH 7.2-7.4, 0.2μm filtered.
Substrate Solution: To achieve best assay results, fresh sub-strate solution is recommended
Substrate stock solution: 10 mg/ml TMB in DMSO
Substrate dilution buffer: 0.05M Na_2HPO_4 and 0.025M citric acid; adjust pH to 5.5
Substrate working solution: For each plate dilute 250 μl sub-strate stock solution in 25ml substrate dilution buffer and then add 80μl 0.75% H_2O_2, mix it well
Stop Solution: 2N H_2SO_4

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PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Keep streptavidin-HRP at 4℃and protect it from prolonged exposure to light. Aliquot all other reagents and store at -20℃ to -70℃ in a manual defrost freezer.

ELISA PROTOCOL

Plate Preparation

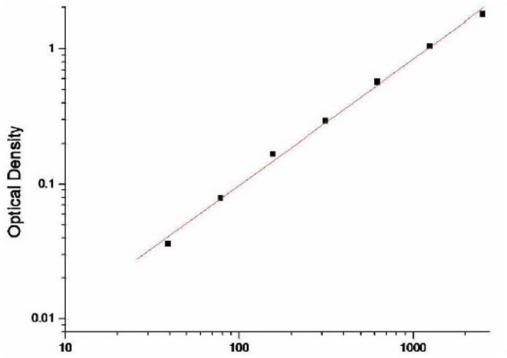
1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100uL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300µl wash buff-er, repeating the process two times for a total of three wash-es. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300µl of blocking buffer to each well. Incubate at room temperature for a minimum of 1hour.

Assay Procedure

1. Add 100 µl of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µl of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100µl of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200 µl of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
8. Add 50µl of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution fac -tor.
Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.



HGFAC concentration(pg/ml)

Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
39.0625	0.036
78.125	0.079
156.25	0.167
312.5	0.295
625	0.572
1250	1.053
2500	1.793

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