HUMAN PLASMA (SOLUBLE) GELSOLIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE GELSOLIN CONCENTRATIONS IN SERUM AND EDTA PLASMA

PRODUCT INFORMATION:

ELISA NAME	HUMAN PLASMA (SOLUBLE) GELSOLIN ELISA
Catalog No.	SK00384-06
Lot No.	
Formulation	96 T
Standard range	6.25-400 ng/mL
Sensitivity	1 ng/mL
Sample require	100 µL
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Sample Type	Serum, EDTA Plasma
Specificity	Human Soluble Gelsolin
Calibration	Human Soluble Gelsolin Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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 Gelsolin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Gelsolin from serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with monoclonal antibody specific for human plasma Gelsolin. The capture antibody can bind to the human plasma Gelsolin in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human plasma Gelsolin is added to the wells. After another washing of the plate, Streptavidin-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 Gelsolin 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. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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
Gelsolin Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human Gelsolin.	**384-06-01**	**1 plate**
Gelsolin Standard – 800 ng/vial of recombinant human Gelsolin in a buffered protein base with preservative; lyophilized.	**384-06-02**	**1 vial**
Detection Antibody – 1.2mL/vial, 10-fold concentrate of a purified antibody biotinylated against soluble human Gelsolin with preservative; lyophilized.	**384-06-03**	**1 vial**
Positive Control – one vial of recombinant soluble human Gelsolin; lyophilized.	**384-06-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µl/vial, 100-fold concentrated solution of Streptavidin-HRP Conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M 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 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, positive control and Detection Antibody concentrated stock solution SHOULD BE STORED at -20° C or -70° C for up to 1 week. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to

8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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) 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples may require 250 ~ 1000 dilution. A suggested 10-fold dilution is 10 µL sample + 90 µL Dilution Buffer. A suggested 250-fold dilution is 10 µL 10-fold diluted sample + 240 µL Dilution Buffer. A suggested 500-fold dilution is 5 µL 100-fold diluted sample + 245 µL Dilution Buffer.
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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Gelsolin Standard - Reconstitute the **Gelsolin** standard with 1 mL of Dilution Buffer (DB01). This reconstitution produces a stock solution of 800 ng/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 #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **400 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 ml	800 ng/ml
# 1	250 µl of stock	250 µl	400 ng/ml
# 2	250µl of 1	250µl	200 ng/ml
# 3	250µl of 2	250µl	100 ng/ml
# 4	250µl of 3	250µl	50 ng/ml
# 5	250µl of 4	250µl	25 ng/ml
# 6	250µl of 5	250µl	12.5 ng/ml
# 7	250µl of 6	250µl	6.25 ng/ml

Positive Control - Reconstitute the Positive Control with 1 mL of **Dilution Buffer (DB01)** to prepare working solution.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Dilution Buffer (DB01)** to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of **Dilution Buffer (DB01)** into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of **Dilution Buffer (DB01)** to prepare working solution. ***Note:*** *1x working solution*

of Streptavidin HRP Conjugate should be used within a few days ***(protect from light)****.*

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 4-5 minute on microplate shaker at room temperature. **Protect from light.**
11. 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.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, 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. The data may be linearized by plotting the log of the Gelsolin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

Calculation of samples with a concentration exceeding that of standard 400 ng/ml may result in inaccurate, low human Gelsolin levels. Such samples require further external predilution according to expected human Gelsolin values with Dilution Buffer in order to precisely quantify the actual human Gelsolin level.

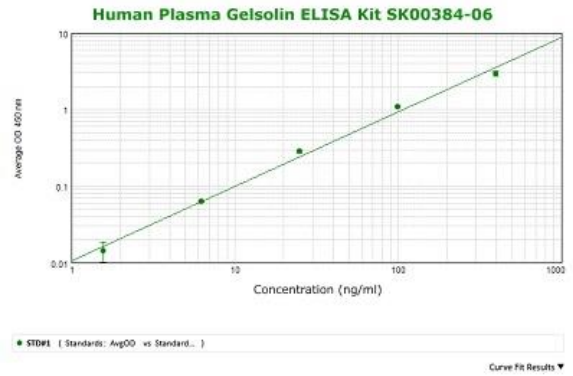
SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human Soluble Gelsolin	100%
Human S100A6	0
Human CRP	0
Human Fetuin A	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 (CORRECTED)
Blank	0 (0.074)
6.25	0.064
12.5	0.120
25	0.241
50	0.493
100	1.024
200	1.928
400	2.910



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 100 µl of standard dilutions, samples, or positive control to the 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 90 mintues on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 40 min on the plate shaker at RT. **Protect from light**.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 4-5 min on the plate shaker at RT. **Protect from light**.
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.