BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human Mesothelin

Cat. No. 438607

ELISA Kit for Accurate Quantitation of Human Mesothelin from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.

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Introduction:

Human mesothelin, also known as CAK1 and MSLN, is a cell-surface protein expressed on normal mesothelial cells lining the pleura, pericardium, and peritoneum. It is expressed as a 70 kD pre-cursor protein with a furin cleavage site, which produces two products: 1) megakaryocyte potentiating factor (MPF) (30 kD); and 2) mesothelin (40 kD). MPF is a cytokine which is released. Mesothelin, for the most part, is membrane-bound by a glycosyl-phosphatidyl inositol linkage and has three glycosylation sites. Soluble mesothelin is believed to result from proteolytic cleavage to release it from the membrane linkage, or from alternative splicing of the mesothelin gene.

The normal function of mesothelin is not known. Mice knockouts produced no obvious phenotypes with normal growth and reproductive parameters. However, mesothelin is overexpressed by cancer cells, particularly in mesotheliomas, ovarian adenocarcinomas, pancreatic adenocarcinomas, squamous cell carcinomas, and lung cancers. It binds to MUC16/CA-125, a cell surface mucin and ovarian cancer biomarker, and has been speculated to be involved in peritoneal dissemination of ovarian cancer cells. Mesothelin has also been shown to be elevated in the serum of cancer patients compared to normal. As such, it has become the focus of cancer research in the areas of diagnostics, immunotherapy, and cancer vaccinology. For example, SS1P is a recombinant immunotoxin that targets mesothelin-expressing cancer cells. Its anti-mesothelin Fv domain confers specificity, while its Pseudomonas exotoxin domain results in cell death. This potential drug has already finished Stage I clinical trials.

BioLegend's LEGEND MAX™ Human Mesothelin ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human Mesothelin capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-human Mesothelin antibody. This kit is specifically designed for the accurate protein quantitation of human mesothelin from cell culture supernatant, serum, plasma and other biological fluids. It is analytically validated with ready-to-use reagents.

Tel: 858-768-5800

Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-human Mesothelin Pre-coated 96-well Strip Micro-plate	1 plate	5 plates		79578
Human Mesothelin Detection Antibody	1 bottle	5 bottles	12 mL	79579
Human Mesothelin Standard	1 vial	5 vials	lyophilized	79589
Avidin-HRP A	1 bottle	5 bottles	12 mL	79131
Assay Buffer A	2 bottle	10 bottles	25 mL	78232
Wash Buffer (20X)	1 bottle	5 bottles	50 mL	78233
Substrate Solution F	1 bottle	5 bottles	12 mL	79132
Stop Solution	1 bottle	5 bottles	12 mL	79133
Plate Sealers	4 sheets	20 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP A</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human Mesothelin Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAXKit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. It is recommended that serum and plasma samples be diluted 20-fold in Assay Buffer A due to high endogenous levels. For example, take 10 µL of sample and dilute it in 190 µL of Assay Buffer A.

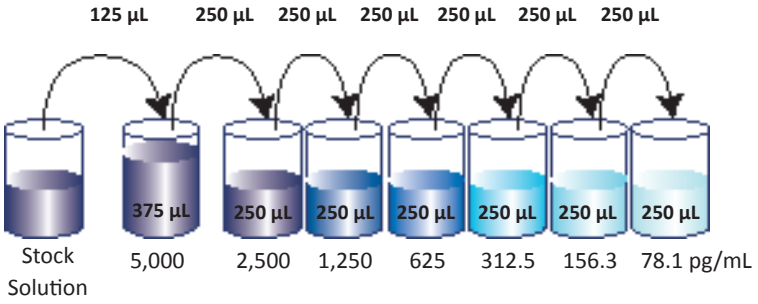
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 5,000 pg/mL top standard by diluting 125 µL of the standard stock solution in 375 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 5,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human mesothelin standard concentrations in the tubes are 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.3 pg/mL and 78.1 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human Mesothelin Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

11. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

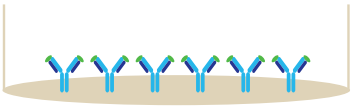
13. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human Mesothelin should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

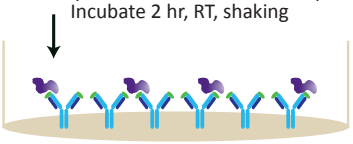
15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

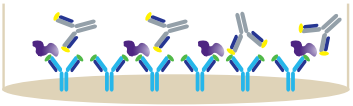
1. Wash 4 times
Add 50 µL Assay Buffer A



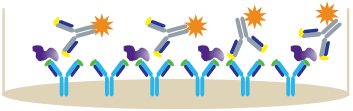
2. Add 50 µL diluted standards or samples
Incubate 2 hr, RT, shaking



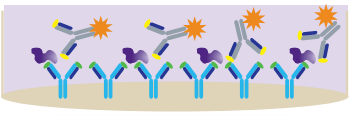
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



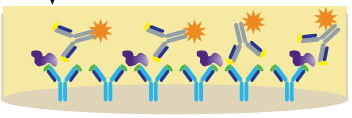
4. Wash 4 times
Add 100 µL Avidin-HRP A solution
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 15 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

Calculation of Results:

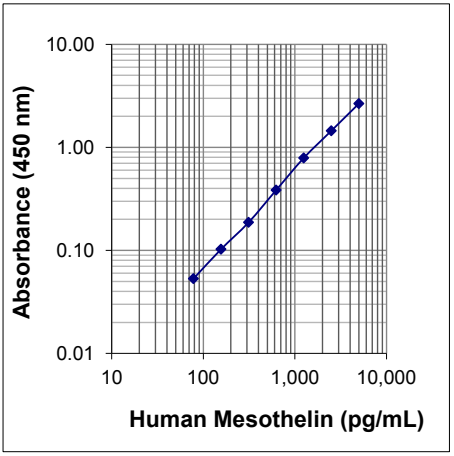
The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration 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 cytokine concentration.

Because serum/plasma samples have been diluted prior to the assay, their measured concentrations must be multiplied by the dilution factor.

If cell culture supernatant samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze 24 human recombinant cytokines/chemokines, each at up to 50 ng/mL.

Human	β_2-Microglobulin, CCL20, CD137, CD40L, Cystatin C, EGF, Eotaxin, FGF-Basic, GADPH, G-CSF, GM-CSF, ICAM-1, IP-10,Lipocalin-2, M-CSF, MCP-1, MCP-3, MIP1-α, Perforin, RANTES, SDF1-α, sFasL,TIM-1, VCAM-1

Sensitivity: The minimum detectable concentration is approximately 35.1 pg/mL (average of 20.7 +/- 7.2 pg/mL in 5 assays).

Recovery: Recombinant human mesothelin, at concentrations of 2500, 625, and 156.3 pg/mL, was spiked into 20-fold dilutions of 10 human serum samples and then analyzed with the LEGEND MAX™ Human Mesothelin ELISA Kit. On average, 98.2% of the protein was recovered from the serum samples.

Linearity: 20-fold dilutions of five human serum samples were further diluted with Assay Buffer A to produce samples with values within the dynamic range and then assayed with the kit to determine the dilution linearity. The linearity of dilution ranged from 88.7 to 123.4%. On average, 98.9% linearity of dilution was observed.

Intra-Assay Precision: Two samples with different mesothelin concentrations were tested with 11 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	11	11
Mean Concentration (pg/mL)	2302.5	267.9
Standard Deviation	27.8	7.2
% CV	1.2	2.7

Inter-Assay Precision: Two samples with different concentrations of mesothelin were assayed in six independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	6	6
Mean Concentration (pg/mL)	2408.2	270.5
Standard Deviation	203.9	19.7
% CV	8.5	7.3

Biological Samples:

Serum - Nineteen human serum samples were assayed for basal levels of human mesothelin. All samples resulted in detectable concentrations, ranging from 12.9-49.5 ng/mL.

Plasma - Eighteen human plasma samples were assayed for basal levels of human mesothelin. All samples resulted in detectable concentrations, ranging from 10.2-90.3 ng/mL.

Cell Culture Supernatant - Human SW480 cells (human colon adenocarcinoma cells), human H1299 cells (human non-small lung carcinoma cells), and human A549 cells (human lung adenocarcinoma cells) were plated in a 6-well plate at 80% confluency. Supernatants were removed at various days and assayed for mesothelin. There was a time-depen-

dent increase in human mesothelin from SW480 cells with the highest level at 15.3 ng/mL on day 7. There was minimal amount of human mesothelin detected from A549 cells (~100 pg/mL) through day 7. There was no detectable human mesothelin from H1299 cells by day 7.

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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