Quantikine® ELISA

Human Mesothelin Immunoassay

Catalog Number DMSLN0

For the quantitative determination of human Mesothelin concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Mesothelin, also known as CAK1 and ERC, is a protein that is mainly expressed in the mesothelial cells lining the pleura, pericardium, and peritoneum. The Mesothelin gene encodes a precursor protein of 70 kDa that is cleaved into two products, including megakaryocyte potentiating factor (amino acids (aa) 37-286) and Mesothelin (aa 296-606). Megakaryocyte potentiation factor is a secreted protein of about 32 kDa, and it functions as a cytokine that can stimulate colony formation in bone marrow megakaryocytes. Mesothelin is a 40 kDa glycosylphosphatidylinositol-anchored cell-surface protein (1-4). Multiple transcription variants of Mesothelin, as a result of alternative splicing, have been described. Variant 1 is primarily expressed at the surface of the cells and can be shed (5-8). Compared to variant 1, variant 2 has an eight aa insertion between aa 409-416 and variant 3 differs from aa 601 to 630 (9). Variant 1 is the predominant form that is expressed by both normal and tumor cells, while variants 2 and 3 exist at low levels (9).

The biological functions of Mesothelin remain unclear. Bera *et al.* generated knockout mice in which the Mesothelin gene was inactivated. They found that these mice did not have any detectable abnormal phenotypes, and they produced offspring normally, suggesting that Mesothelin is not essential for growth and development (10). Other studies have reported that cells transfected with Mesothelin were more difficult to remove from the culture dishes than nontransfected cells. This indicates that Mesothelin might function as an adhesion molecule (1). This notion is further supported by the observation that Mesothelin is able to bind to CA125/MUC16 with a high affinity and that this interaction mediates cell adhesion (11, 12). Mesothelin may thus play an important role in the peritoneal spread of ovarian cancer (13). The expression of Mesothelin is modulated by the Wnt proteins. In mouse mammary epithelial cells, Mesothelin is up-regulated by Wnt-1, while down-regulated by Wnt-5a (14). Under physiological conditions, the expression of Mesothelin is limited, however, overexpression of Mesothelin has been described in many types of malignancies, such as mesothelioma, ovarian cancer, and pancreatic cancer (15-17). Further studies have suggested that this cancer-specific overexpression of Mesothelin might be attributed to an upstream transcription enhancer (18). Mesothelin is detectable in many biological fluids, such as serum and urine. It has been demonstrated that quantification of Mesothelin in these biological fluids may serve as a useful biomarker for cancer diagnosis, prognosis, and monitoring response to therapy in patients with mesothelioma and ovarian cancer (19, 20). Furthermore, due to its low expression in normal tissues and high expression in tumor cells, Mesothelin is an attractive candidate for cancer immunotherapy. Additionally, Mesothelin can elicit an autoimmune response in cancer patients. These therapies include agents that target cell surface Mesothelin or elicit an immune response against Mesothelin (21-23).

The Quantikine Human Mesothelin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Mesothelin in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human Mesothelin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Mesothelin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Mesothelin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Mesothelin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Mesothelin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Mesothelin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Mesothelin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Mesothelin Microplate	894003	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Mesothelin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Mesothelin Conjugate	894004	21 mL of monoclonal antibody specific for human Mesothelin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Mesothelin Standard	894005	100 ng of recombinant human Mesothelin in a buffered protein solution with preservatives; lyophilized.	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-20 Concentrate	895346	21 mL of a concentrated buffered protein base with preservatives. Use diluted 1:5 in this assay.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Mesothelin Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum, plasma, and saliva samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-20 (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

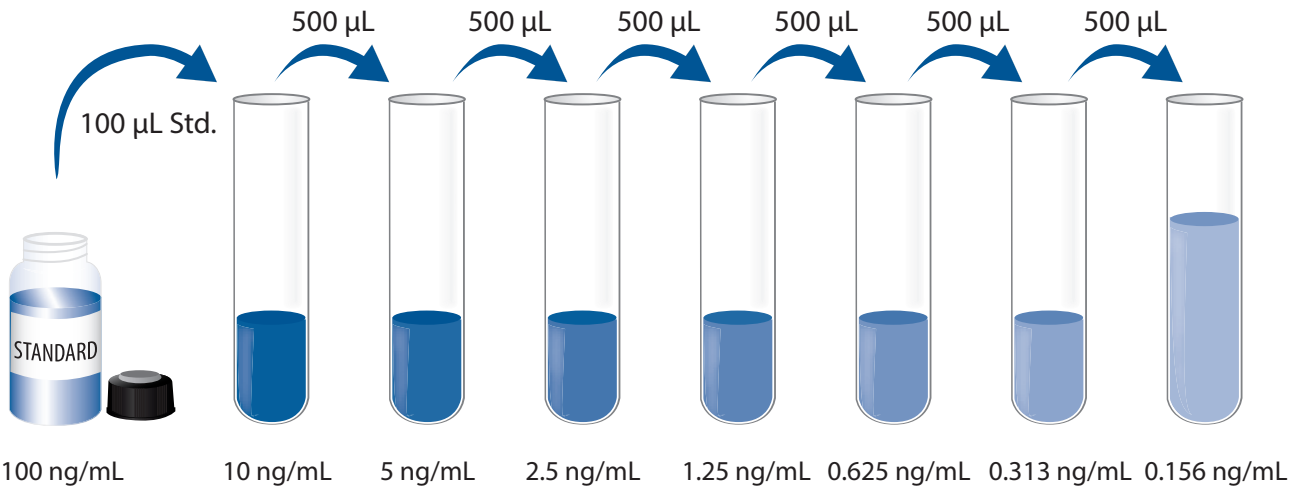
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-20 (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5-20 to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5-20 (diluted 1:5).

Human Mesothelin Standard - Reconstitute the Human Mesothelin Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-20 (diluted 1:5) into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-20 (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1S to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 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 200 µL of Human Mesothelin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

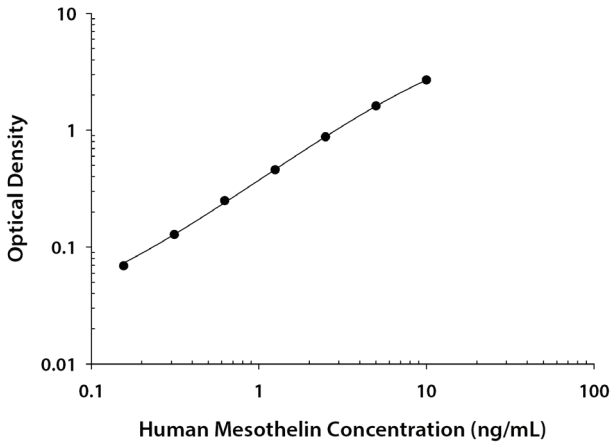
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 human Mesothelin 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.

TYPICAL DATA

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



(ng/mL)	O.D.	Average	Corrected
0	0.021 0.021	0.021	—
0.156	0.088 0.091	0.090	0.069
0.313	0.148 0.150	0.149	0.128
0.625	0.269 0.273	0.271	0.250
1.25	0.470 0.490	0.480	0.459
2.5	0.894 0.901	0.898	0.877
5	1.619 1.642	1.631	1.610
10	2.659 2.756	2.708	2.687

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.830	2.56	5.54	0.910	2.80	5.94
Standard deviation	0.034	0.082	0.193	0.057	0.130	0.360
CV (%)	4.1	3.2	3.5	6.3	4.6	6.1

RECOVERY

The recovery of human Mesothelin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	104	91-113%
Serum* (n=4)	99	92-114%
EDTA plasma* (n=4)	97	88-109%
Heparin plasma* (n=4)	97	86-112%
Saliva* (n=4)	102	91-112%
Urine (n=4)	103	86-115%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human Mesothelin ranged from 0.005-0.022 ng/mL. The mean MDD was 0.010 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Mesothelin were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva* (n=4)	Urine (n=4)
1:2	Average % of Expected	103	98	99	97	99	100
	Range (%)	101-105	91-104	94-103	91-103	98-101	93-106
1:4	Average % of Expected	104	98	97	91	100	99
	Range (%)	103-106	91-104	93-98	88-93	90-108	97-100
1:8	Average % of Expected	107	98	95	93	104	106
	Range (%)	105-109	92-104	94-96	89-98	91-115	100-112
1:16	Average % of Expected	108	95	95	90	102	103
	Range (%)	103-114	92-99	91-97	88-92	90-113	90-114

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Mesothelin (amino acids Glu296-Gly580, Accession # AAH09272) produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Mesothelin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	18.7	9.63-40.2	7.0
EDTA plasma (n=35)	18.1	9.24-40.8	6.9
Heparin plasma (n=35)	16.4	8.31-44.7	7.2
Saliva (n=10)	14.5	4.72-27.7	7.7
Urine (n=11)	6.57	0.39-22.7	7.1

Cell Culture Supernates:

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Mesothelin, and measured 2.92 ng/mL.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 10% fetal bovine serum and 0.25 g/mL mouse insulin until confluent. An aliquot of the cell culture supernate was removed, assayed for human Mesothelin, and measured 7.92 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Mesothelin.

The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human Mesothelin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CA125/MUC16
CD14
CD48/SLAMF2
MPF/Megakaryocyte Potentiating Factor
ULBP-1
ULBP-3
ULBP-4

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