PRODUCT INFORMATION & MANUAL

Human Mesothelin Valukine™ ELISA

Catalog Number: VAL238

For the quantitative determination of natural and recombinant human Mesothelin concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

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).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

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

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernates, human serum, human plasma, human saliva and human urine.
- 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, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
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

B. 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
Human Serum* (n=4)	99	92-114
Human EDTA plasma* (n=4)	97	88-109
Human Heparin plasma* (n=4)	97	86-112
Human Saliva* (n=4)	102	91-112
Human Urine (n=4)	103	86-115

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

C. 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.

D. 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.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Mesothelin and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=2)	Human Serum* (n=4)	Human EDTA plasma* (n=4)	Human Heparin plasma* (n=4)	Human Saliva* (n=4)	Human 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.

F. SAMPLE VALUES

Human 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)
Human Serum (n=35)	18.7	9.63-40.2	7.0
Human EDTA plasma (n=35)	18.1	9.24-40.8	6.9
Human Heparin plasma (n=35)	16.4	8.31-44.7	7.2
Human Saliva (n=10)	14.5	4.72-27.7	7.7
Human 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.

G. SPECIFICITY

This assay recognizes natural and recombinant human Mesothelin.

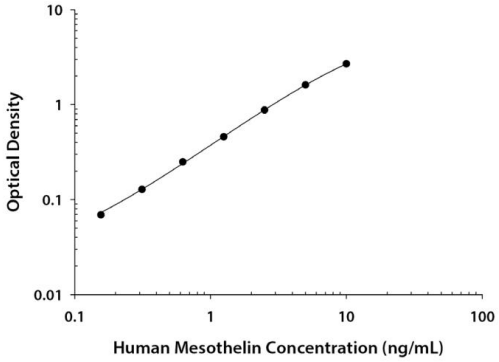
The factors listed below were prepared at 100 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human Mesothelin control were assayed for interference. No significant cross-reactivity or interference was observed.

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

IV. EXPERIMENT

EXAMPLE STANDARD

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
2.50	0.894 0.901	0.898	0.877
5.00	1.619 1.642	1.631	1.610
10.0	2.659 2.756	2.708	2.687

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Mesothelin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Mesothelin	1 plate
Human Mesothelin Conjugate	Solution of antibody against human Mesothelin conjugated to horseradish peroxidase	1 vial
Human Mesothelin Standard	Recombinant human Mesothelin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1S	A buffered protein base	1 vial
Calibrator Diluent Concentrate (5×)/ RD5-20	A 5× concentrated buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="6">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1S</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent Concentrate (5×)/ RD5-20</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. 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 cylinder.
- Horizontal orbital shaker (0.12” orbit) capable of maintaining a speed of 500±50 rpm
- Test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND 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. Samples may require dilution with Calibrator Diluent (1×).

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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

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 × g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

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. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Human serum, human plasma and human saliva samples recommend a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

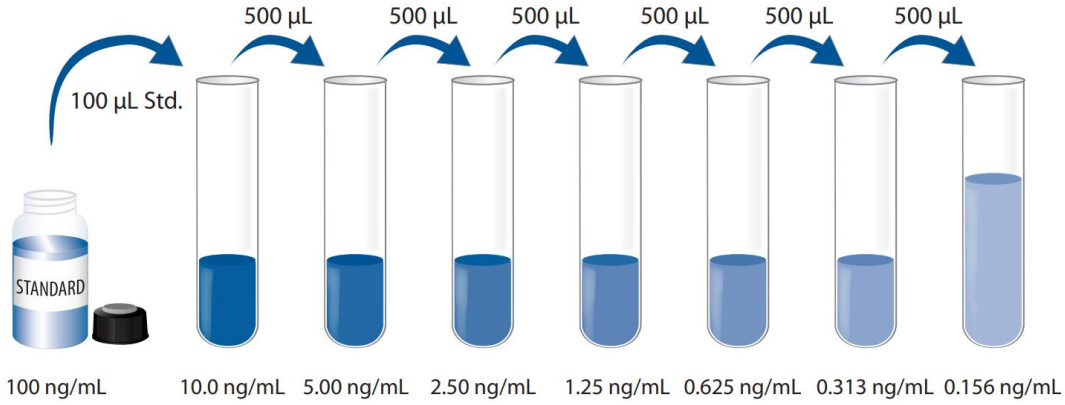
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator

Diluent (1×).

Human Mesothelin Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human Mesothelin Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 900 µL of Calibrator Diluent (1×) into the 10.0 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 10.0 ng/mL standard serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 ng/mL).



D. 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

VII.ASSAY PROCEDURE

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

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 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 and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.
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 a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 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.
11. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard 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.

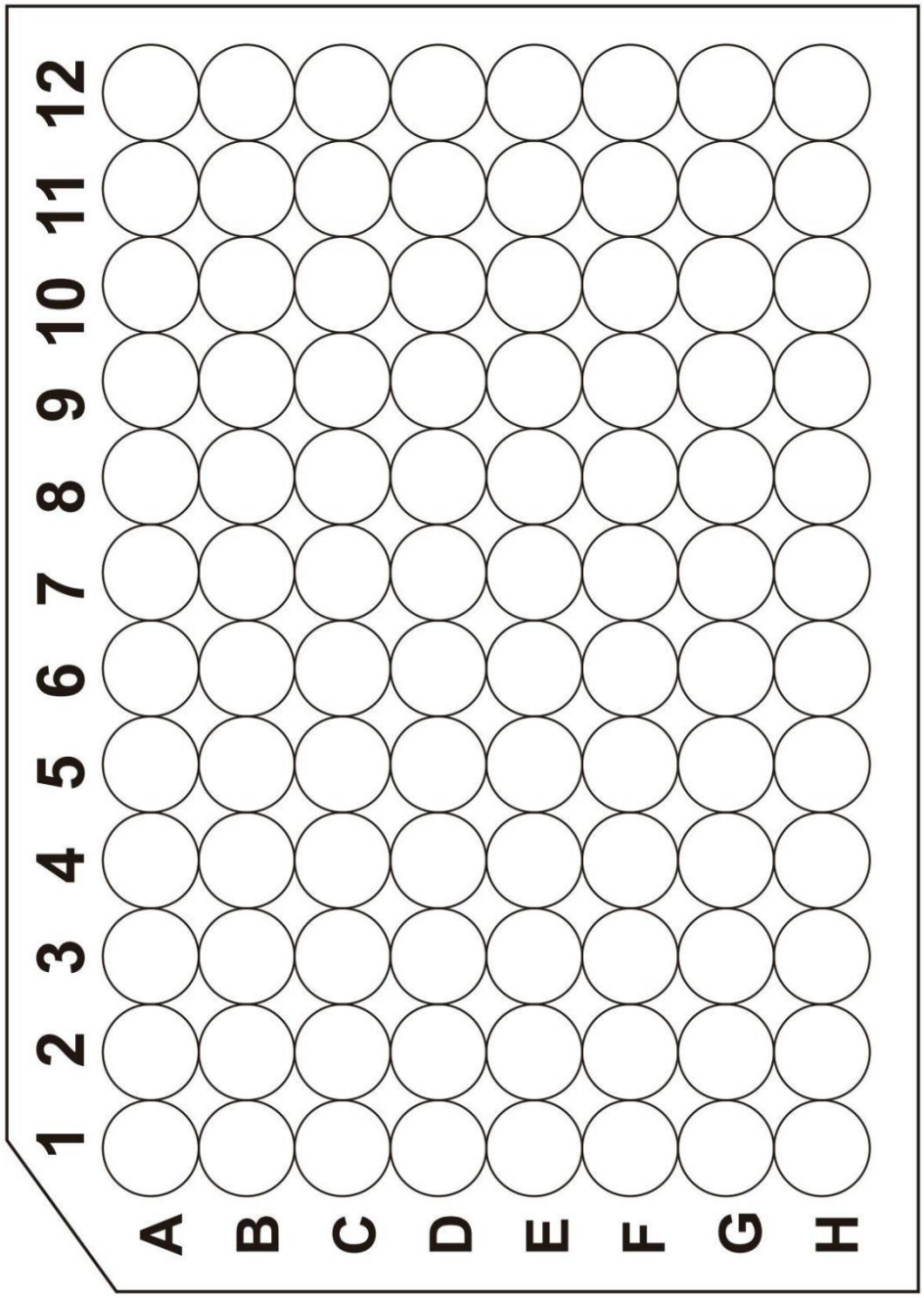
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Chang, K. *et al.* (1996) Proc. Natl. Acad. Sci. USA 93:136.
2. Yamaguchi, N. *et al.* (1994) J. Biol. Chem. 269:805.
3. Chang, K. *et al.* (1992) Int. J. Cancer 50:373.
4. Kojima, T. *et al.* (1995) J. Biol. Chem. 270:21984.
5. Scholler, N. *et al.* (1999) Proc. Natl. Acad. Sci. USA 96:11531.
6. Hellstrom, I. *et al.* (2006) Cancer Epidemiol. Biomarkers Prev. 15:1014.
7. Ho, M. *et al.* (2006) Cancer Epidemiol. Biomarkers Prev. 15:9.
8. Segawa, T. *et al.* (2008) Biochemical and Biophysical Res. Comm. 369:915.
9. Muminova, Z.E. *et al.* (2004) BMC Cancer 4:19.
10. Bera, T. *et al.* (2000) Mol. Cell. Biol. 20:2902.
11. Rump, A. *et al.* (2004) J. Biol. Chem. 279:9190.
12. Gubbels, J.A.A. *et al.* (2006) Mol. Cancer 5:50.
13. Kaneko, O. *et al.* (2009) J. Biol. Chem. 284:3739.
14. Prieve, M.G. *et al.* (2003) BMC Dev. Biol. 3:2.
15. Argni, P. *et al.* (2001) Clin. Cancer Res. 7:3862.
16. Yen, J.M. *et al.* (2006) Clin. Cancer Res. 12:827.
17. Ryu, B. *et al.* (2002) Cancer Res. 62:819.
18. Hucl, T. *et al.* (2007) Cancer Res. 67:9055.
19. Robinson, B.W. *et al.* (2003) Lancet 362:1612.
20. Hassan, R. *et al.* (2006) Clin. Cancer Res. 12:447.
21. Hassan, R. *et al.* (2008) Eur. J. Cancer 44:46.
22. Ho, J.M. *et al.* (2005) Clin. Cancer Res. 11:3814.
23. Thomas, A.M. *et al.* (2004) J. Exp. Med. 200:297.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Mesothelin Valukine™ ELISA 试剂盒

目录号：VAL238

适用于定量检测天然和重组人 Mesothelin 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景

间皮素（Mesothelin）又称 CAK1 和 ERC，是一种主要在胸膜、心包和腹膜的间皮细胞中表达的蛋白质。Mesothelin 基因编码一种 70 kDa 的前体蛋白，该蛋白被裂解为两种产物，包括巨核细胞增效因子（氨基酸（aa） 37-286）和 Mesothelin（aa 296-606）。巨核细胞增效因子是一种约 32 kDa 的分泌蛋白，它是一种细胞因子，可刺激骨髓巨核细胞的集落形成。Mesothelin 是一种 40 kDa 的糖基磷脂酰肌醇锚定细胞表面蛋白（1-4）。Mesothelin 有多种转录变体，这是替代剪接的结果。变体 1 主要在细胞表面表达，可以脱落（5-8）。与变体 1 相比，变体 2 在 aa 409-416 之间插入了 8 个 aa，而变体 3 在 aa 601-630 之间有所不同（9）。变体 1 是主要形式，在正常细胞和肿瘤细胞中均有表达，而变体 2 和变体 3 的表达量较低（9）。

Mesothelin 的生物功能仍不清楚。Bera 等人在小鼠上进行了基因敲除，使 Mesothelin 基因失活。他们发现，这些小鼠没有任何可检测到的异常表型，而且它们能正常生育后代，这表明 Mesothelin 不是生长和发育所必需的物质（10）。其他研究报告称，与未转染 Mesothelin 的细胞相比，转染 Mesothelin 的细胞更难从培养皿中移除。这表明 Mesothelin 可能具有粘附分子的功能（1）。Mesothelin 能以高亲和力与 CA125/MUC16 结合，并通过这种相互作用介导细胞粘附，这一观察结果进一步支持了这一观点（11, 12）。因此，Mesothelin 可能在卵巢癌的腹膜扩散中发挥重要作用（13）。Mesothelin 的表达受 Wnt 蛋白调节。在小鼠乳腺上皮细胞中，Mesothelin 受 Wnt-1 上调，受 Wnt-5a 下调（14）。在生理条件下，Mesothelin 的表达是有限的，但在许多类型的恶性肿瘤中，如间皮瘤、卵巢癌和胰腺癌中，Mesothelin 都被描述为过表达（15-17）。进一步的研究表明，这种癌症特异性的 Mesothelin 过表达可能归因于上游转录增强子（18）。在血清和尿液等许多生物液体中都能检测到间皮素。研究表明，对这些生物液体中的 Mesothelin 进行定量分析可作为一种有用的生物标记物，用于间皮瘤和卵巢癌患者的癌症诊断、预后和治疗反应监测（19, 20）。此外，由于 Mesothelin 在正常组织中的低表达量和在肿瘤细胞中的高表达量，Mesothelin 是一种有吸引力的癌症免疫疗法候选药物。此外，间皮素还能引起癌症患者的自身免疫反应。这些疗法包括靶向细胞表面 Mesothelin 或引起针对 Mesothelin 的免疫反应的制剂（21-23）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Mesothelin抗体包被于微孔板上，样品和标准品中的人Mesothelin会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人Mesothelin检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断**。
- 该试剂盒适用于细胞培养上清样本、人血清、人血浆、人唾液和人尿液。
- 请在试剂盒有效期内使用。
- 不同试剂盒及不同批号试剂盒的组分不能混用。
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（ng/mL）	0.830	2.56	5.54	0.910	2.80	5.94
标准差	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

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Mesothelin，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	104	91-113
人血清*（n=4）	99	92-114
人 EDTA 血浆*（n=4）	97	88-109
人肝素血浆*（n=4）	97	86-112
人唾液*（n=4）	102	91-112
人尿液（n=4）	103	86-115

*样品在分析前按照样品制备部分的指示进行稀释。

C. 灵敏度

评估 20 项检测，人 Mesothelin 的最小可检测剂量（MDD）范围为 0.005-0.022 ng/mL，平均 MDD 为 0.010 ng/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组人Mesothelin（氨基酸序列Glu296-Gly580，登录号AAH09272）校正。

E. 线性

不同的样本中含有或掺入高浓度的人Mesothelin，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清液 (n=2)	人血清* (n=4)	人EDTA血浆* (n=4)	人肝素血浆* (n=4)	人唾液* (n=4)	人尿液（n=4）
1:2	平均值/期待值（%）	103	98	99	97	99	100
	范围（%）	101-105	91-104	94-103	91-103	98-101	93-106
1:4	平均值/期待值（%）	104	98	97	91	100	99
	范围（%）	103-106	91-104	93-98	88-93	90-108	97-100
1:8	平均值/期待值（%）	107	98	95	93	104	106
	范围（%）	105-109	92-104	94-96	89-98	91-115	100-112
1:16	平均值/期待值（%）	108	95	95	90	102	103
	范围（%）	103-114	92-99	91-97	88-92	90-113	90-114

*样品在分析前按照样品制备部分的指示进行稀释。

F. 样本预值

人血清/血浆/唾液/尿液 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人 Mesothelin。本研究中使用的供体没有病史。

样本类型	平均值（ng/mL）	范围（ng/mL）	标准差（ng/mL）
人血清（n=35）	18.7	9.63-40.2	7.0
人EDTA血浆（n=35）	18.1	9.24-40.8	6.9
人肝素血浆（n=35）	16.4	8.31-44.7	7.2
人唾液（n=10）	14.5	4.72-27.7	7.7
人尿液（n=11）	6.57	0.39-22.7	7.1

细胞培养上清：

将HepG2人肝细胞癌细胞在有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的DMEM中培养至汇合。取细胞培养上清液，测定人Mesothelin，测定值为2.92 ng/mL。

将OVCAR-3人卵巢癌细胞在有10%胎牛血清和0.25 g/mL小鼠胰岛素的RPMI中培养至汇合。取细胞培养上清液，测定人Mesothelin，测定值为7.92 ng/mL。

G. 特异性

检测方法识别天然和重组人Mesothelin。

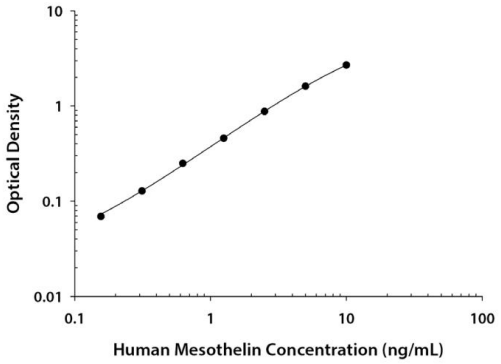
以下列出的因子在标准品稀释液（1×）中以100 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人Mesothelin对照品中以100 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

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

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(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
2.50	0.894 0.901	0.898	0.877
5.00	1.619 1.642	1.631	1.610
10.0	2.659 2.756	2.708	2.687

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Mesothelin Microplate	包被抗人Mesothelin抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Mesothelin Conjugate	酶标检测抗人Mesothelin抗体	1瓶
Human Mesothelin Standard	重组人Mesothelin标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1S	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5-20	浓缩的标准品稀释液（5×），用于稀释标准品和样品	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1S</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5-20</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；
2-8°C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 50 mL和500 mL量筒
- 水平振荡器（0.12”轨道），转速：500±50 rpm
- 用于稀释标准品和样品的管子

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固30分钟，然后在1000 × g的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用肝素或EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

唾液 - 将唾液收集到管中，以10000 × g离心5分钟。收集水层，立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

尿液 - 无菌采集当日首次尿液（中段尿），直接排入无菌容器中。离心去除颗粒物质，立即检测或分装后储存于≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）进行稀释。

B. 样品准备

人血清、人血浆和人唾液样本建议稀释 10 倍。建议的 10 倍稀释方式为：50 µL 的样本+450 µL 的标准品稀释液（1×）。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

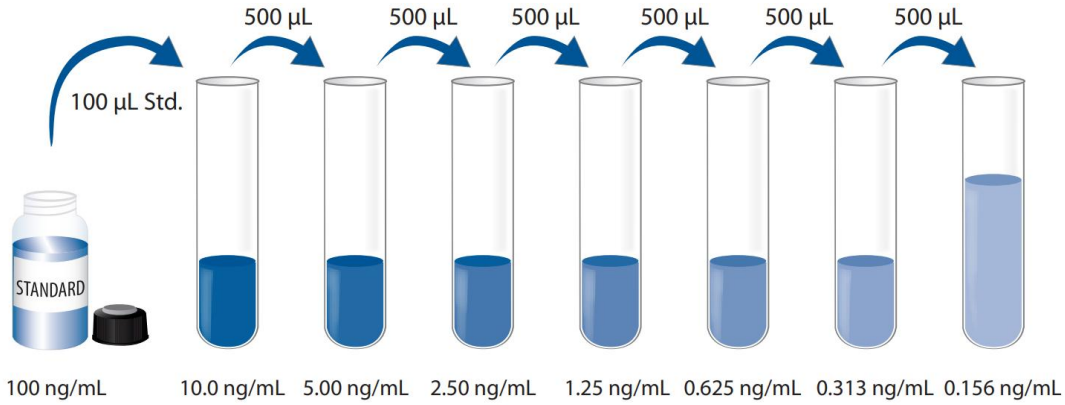
标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Mesothelin标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶人Mesothelin标准品，得到浓度为100 ng/mL标准品储备母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将900 µL标准品稀释液（1×）移入10.0 ng/mL的管中，其余管中加入500 µL 标

准品稀释液（1×）。使用标准品储备母液稀释（如下）。在下次转移之前，将每个管子彻底混合。10 ng/mL作为标准曲线的最高点。标准品稀释液（1×）作为标准曲线的零点（0 ng/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫。
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽。
- 建议15分钟内完成一块板的上样。
- 每次孵育时，正确使用封板膜可保证结果的准确性。
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色。
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII.操作步骤

使用前，将所有其他试剂和样品放置于室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品。
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口。
3. 向每个孔中加入100 µL的检测液RD1S。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置。
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体。
6. 在每个微孔内加入200 µL人Mesothelin酶标检测抗体。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确。
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来建立标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人Mesothelin浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Chang, K. *et al.* (1996) Proc. Natl. Acad. Sci. USA 93:136.
2. Yamaguchi, N. *et al.* (1994) J. Biol. Chem. 269:805.
3. Chang, K. *et al.* (1992) Int. J. Cancer 50:373.
4. Kojima, T. *et al.* (1995) J. Biol. Chem. 270:21984.
5. Scholler, N. *et al.* (1999) Proc. Natl. Acad. Sci. USA 96:11531.
6. Hellstrom, I. *et al.* (2006) Cancer Epidemiol. Biomarkers Prev. 15:1014.
7. Ho, M. *et al.* (2006) Cancer Epidemiol. Biomarkers Prev. 15:9.
8. Segawa, T. *et al.* (2008) Biochemical and Biophysical Res. Comm. 369:915.
9. Muminova, Z.E. *et al.* (2004) BMC Cancer 4:19.
10. Bera, T. *et al.* (2000) Mol. Cell. Biol. 20:2902.
11. Rump, A. *et al.* (2004) J. Biol. Chem. 279:9190.
12. Gubbels, J.A.A. *et al.* (2006) Mol. Cancer 5:50.
13. Kaneko, O. *et al.* (2009) J. Biol. Chem. 284:3739.
14. Prieve, M.G. *et al.* (2003) BMC Dev. Biol. 3:2.
15. Argni, P. *et al.* (2001) Clin. Cancer Res. 7:3862.
16. Yen, J.M. *et al.* (2006) Clin. Cancer Res. 12:827.
17. Ryu, B. *et al.* (2002) Cancer Res. 62:819.
18. Hucl, T. *et al.* (2007) Cancer Res. 67:9055.
19. Robinson, B.W. *et al.* (2003) Lancet 362:1612.
20. Hassan, R. *et al.* (2006) Clin. Cancer Res. 12:447.
21. Hassan, R. *et al.* (2008) Eur. J. Cancer 44:46.
22. Ho, J.M. *et al.* (2005) Clin. Cancer Res. 11:3814.
23. Thomas, A.M. *et al.* (2004) J. Exp. Med. 200:297.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置