ab204528 – Mesothelin Mouse SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of Mesothelin in mouse serum, plasma, cell culture supernatants and, cell and tissue extract samples.

This product is for research use only and is not intended for diagnostic use.

Version 4a Last Updated 11 April 2024

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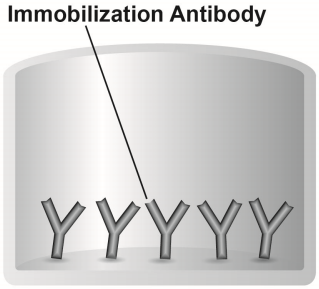
1. BACKGROUND

Abcam's Mesothelin *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Mesothelin protein in mouse serum, plasma, cell culture supernatants and, cell and tissue extract samples.

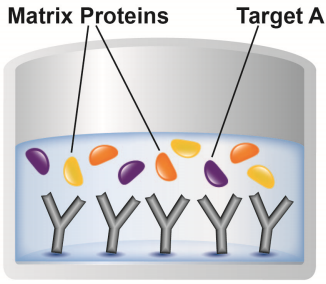
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Mesothelin is expressed as a much larger, 70 kDa precursor, which is cleaved into two functional proteins, Megakaryocyte potentiating factor and Mesothelin. Mesothelin is a glycosylated cell-surface protein, anchored to the plasma membrane via its GPI anchor. Membrane-anchored forms of Mesothelin may play a role in cellular adhesion.

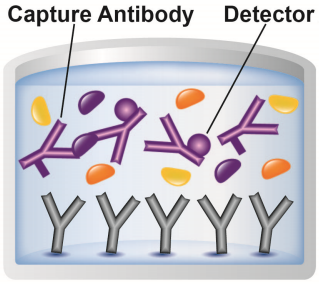
2. ASSAY SUMMARY



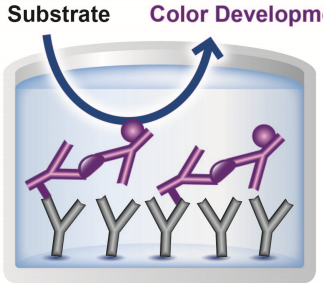
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Mouse Mesothelin Capture Antibody	600 µL	+4ºC
10X Mouse Mesothelin Detector Antibody	600 µL	+4ºC
Mouse Mesothelin Lyophilized Recombinant Protein	2 Vials	+4ºC
Antibody Diluent CPI2*	6 mL	+4ºC
10X Wash Buffer PT	20 mL	+4ºC
5X Cell Extraction Buffer PTR	10 mL	+4ºC
50X Cell Extraction Enhancer Solution	1 mL	+4ºC
TMB Substrate	12 mL	+4ºC
Stop Solution	12 mL	+4ºC
Sample Diluent NS	50 mL	+4ºC
Sample Diluent 25BS	20 mL	+4ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+4ºC
Plate Seal	1	+4ºC

***Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data**

equivalent to the original formulation of antibody diluent CPI previously used in this kit.

While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit.

If you have any questions please contact Abcam Scientific Support.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and

performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL 5X Cell Extraction Buffer PTR. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the Leptin standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Leptin standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 11,200 pg/mL **Stock Standard** Solution.

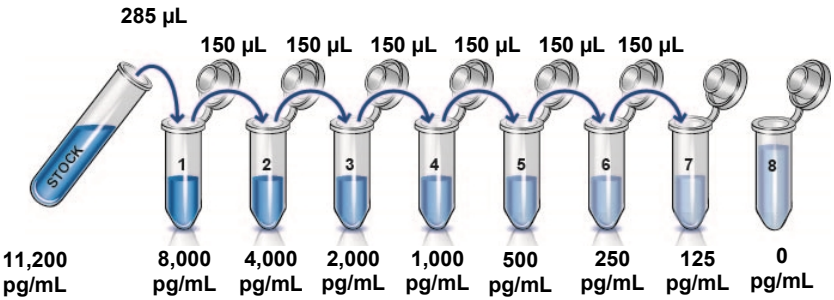
10.1 For **cell culture supernatant and, cell and tissue extract samples** follow this section to prepare the standard.

10.1.1 For **cell culture supernatant** samples, reconstitute the Mesothelin standard by adding Sample Diluent NS by pipette.
For **cell and tissue extract** samples, reconstitute the Mesothelin standard by adding 1X Cell Extraction Buffer PTR by pipette.

10.1.2 Label eight tubes, Standards 1– 8.

10.1.3 For **cell culture supernatant** samples add 114 µL Sample Diluent NS tube number 1 and 150 µL of into numbers 2-8.
For **cell and tissue extract** samples add 114 µL 1X Cell Extraction Buffer PTR tube number 1 and 150 µL into numbers 2-8.

10.1.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



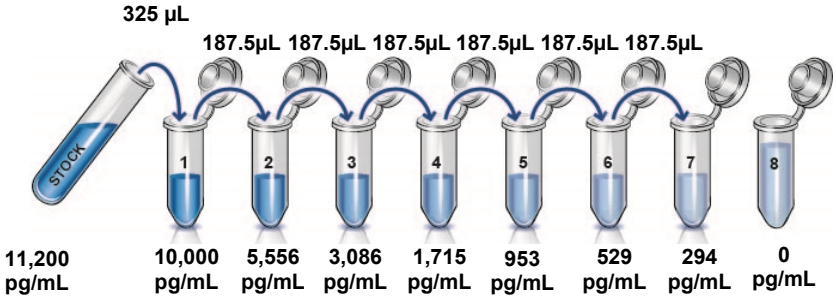
10.2 For **serum and plasma samples** follow this section to prepare the standard.

10.2.1 Reconstitute the Mesothelin standard by adding Sample Diluent 25BS by pipette.

10.2.2 Label eight tubes, Standards 1– 8.

10.2.3 Add 39 µL of Sample Diluent 25BS into tube number 1 and 150 µL of Sample Diluent 25BS into numbers 2-8.

10.2.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Mouse Serum	9 – 50%
Mouse Plasma -Citrate	15 -50%
Mouse plasma - EDTA	15 -50%
C2C12 cell extract	16 – 125 µg/mL

11.1 **Plasma**

Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma samples at least to 50% in Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute serum samples at least to 50% in Sample Diluent 25BS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 **Cell Culture Supernatants**

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute cell culture supernatant samples at least to 50% in Sample Diluent NS and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 **Preparation of extracts from cell pellets**

11.4.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture

flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.4.2 Rinse cells twice with PBS.

11.4.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.4.4 Incubate on ice for 20 minutes.

11.4.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.4.6 Transfer the supernatants into clean tubes and discard the pellets.

11.4.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.4.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.5 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

11.5.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.5.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.5.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.5.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.5 Transfer the supernatants into clean tubes and discard the pellets.

11.5.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.6 **Preparation of extracts from tissue homogenates**

11.6.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.6.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.6.3 Incubate on ice for 20 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

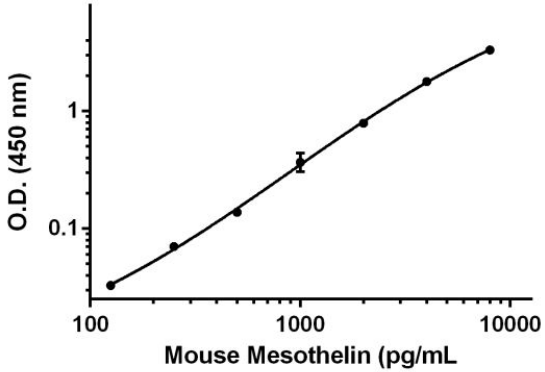
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

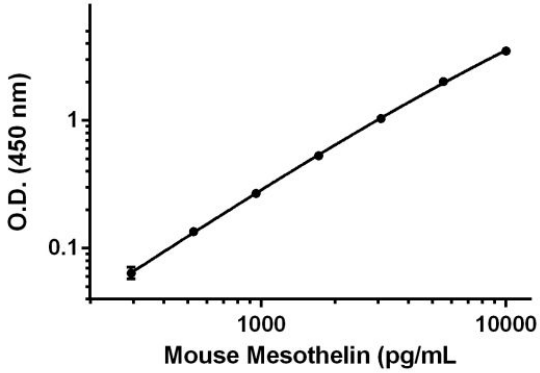
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



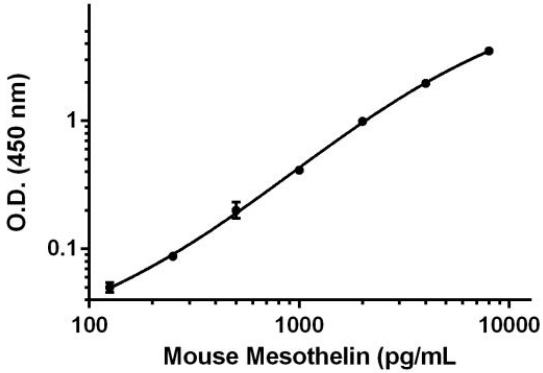
Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.074	0.078	0.076
125	0.107	0.110	0.109
250	0.146	0.147	0.147
500	0.214	0.213	0.214
1,000	0.399	0.495	0.448
2,000	0.862	0.875	0.869
4,000	1.880	1.862	1.871
8,000	3.295	3.505	3.401

Figure 1. Example of Mesothelin standard curve in Sample Diluent NS. The Mesothelin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.066	0.061	0.064
294	0.132	0.123	0.128
529.2	0.198	0.199	0.199
952.6	0.339	0.324	0.332
1,714.7	0.600	0.588	0.594
3,086.4	1.117	1.083	1.100
5,555.6	2.137	2.024	2.081
10,000	3.626	3.505	3.566

Figure 2. Example of Mesothelin standard curve in Sample Diluent 25BS. The Mesothelin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.053	0.060	0.057
125	0.110	0.104	0.107
250	0.146	0.143	0.145
500	0.237	0.278	0.258
1,000	0.461	0.477	0.470
2,000	1.065	1.032	1.049
4,000	1.937	2.109	2.023
8,000	3.502	3.640	3.571

Figure 3. Example of Mesothelin standard curve in 1X Cell Extraction Buffer PTR. The Mesothelin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration. The MDD is dependent on the Sample Diluent buffer used:

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	20	41 pg/mL
Sample Diluent 25BS	25	70 pg/mL
1X Cell Extraction Buffer PTR	28	14 pg/mL

RECOVERY –

For **cell culture supernatant, serum and plasma samples** measurements, three concentrations of mouse recombinant Mesothelin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% 10F RPMI1640 medium	88.5	85.6 – 90.0
25% Mouse Serum	119.6	115.6 – 122.8
50% Mouse Plasma - Citrate	112.1	108.2 – 114.6
50% Mouse Plasma - EDTA	116.3	114.4 – 118.8

For **cell and tissue extract samples** measurements, three concentrations of mouse recombinant Mesothelin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Cell Culture Media	89.2	82.0 - 95.0
10% Human serum	95.4	87.9 – 99.2

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Mesothelin was measured in the following biological samples in a 1.8-fold dilution series in Sample Diluent 25BS.

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Cit.)	50% Mouse Plasma (EDTA)
Undiluted	pg/mL	1,552.21	780.45	1,053.22
	% Expected value	**100**	**100**	**100**
1.8	pg/mL	876.47	583.44	578.83
	% Expected value	**101.6**	**134.6**	**98.9**
3.24	pg/mL	492.54	322.71	226.86
	% Expected value	**102.8**	**133.9**	**77.5**
5.832	pg/mL	282.56	146.65	NL
	% Expected value	**106.2**	**109.6**	**NL**

NL – Non-Linear

Native Mesothelin was measured in the following biological samples in a 2-fold dilution series in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	125 µg/mL C2C12 Cell Extract
Undiluted	pg/mL	823.57
	% Expected value	**100**
2	pg/mL	426.73
	% Expected value	**103.6**
4	pg/mL	232.64
	% Expected value	**113**
8	pg/mL	128.02
	% Expected value	**124.4**

Recombinant Mouse Mesothelin was spiked into the following biological samples and diluted in a 1.8-fold dilution series in Sample Diluent 25BS.

Dilution Factor	Interpolated value	50% Mouse Serum	28% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)
Undiluted	pg/mL	10,585.70	4,980.98	11,556.10
	% Expected value	**100.0**	**100.0**	**100.0**
1.8	pg/mL	5,767.23	2,497.45	6,813.03
	% Expected value	**98.1**	**90.3**	**106.1**
3.24	pg/mL	2,965.84	1,266.21	3,318.19
	% Expected value	**90.8**	**82.4**	**93.0**
5.832	pg/mL	1,679.10	675.69	1,732.52
	% Expected value	**92.5**	**79.1**	**87.4**
10.498	pg/mL	936.20	418.39	973.24
	% Expected value	**92.8**	**88.2**	**88.4**
18.896	pg/mL	539.46	269.91	569.02
	% Expected value	**96.3**	**102.4**	**93.0**
34.013	pg/mL	267.89	NL	325.69
	% Expected value	**86.1**	**NL**	**95.9**

NL – Non-Linear

Recombinant Mouse Mesothelin was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% 10F RPMI 1640 medium
Undiluted	pg/mL	1,902.45
	% Expected value	**100**
2	pg/mL	1,139.78
	% Expected value	**119.8**
4	pg/mL	641.13
	% Expected value	**134.8**
8	pg/mL	290.39
	% Expected value	122.1
16	pg/mL	118.66
	% Expected value	**99.8**

PRECISION –

Mean coefficient of variations of interpolated values from 2 concentrations of Mouse plasma (citrate) within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	5	3
CV (%)	8.3	9.8

Serum/Plasma. Pooled female mouse serum and plasma samples were measured in three different dilutions in duplicates for Mesothelin concentrations using this kit. Means of interpolated sample values adjusted to sample dilution are shown in pg of Mesothelin per mL of serum/plasma in the table below.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse Serum	3,213	3,104 – 3,379
Mouse Plasma - Citrate	2,104	1,561 – 2,651
Mouse Plasma - EDTA	1,941	1,633 – 2,106

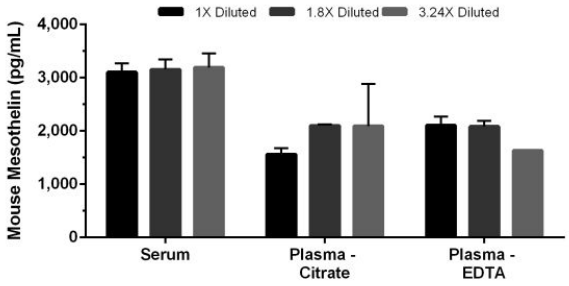


Figure 4. Interpolated concentrations of Mesothelin in mouse serum and plasma samples. The concentrations of Mesothelin were measured in duplicates, interpolated from the Mesothelin standard curve and corrected for sample dilution. Note that 1X Diluted samples were pre-diluted to 50%. The

interpolated, dilution factor-corrected values are plotted in pg of Mesothelin per mL of neat sample (mean +/- SD, n=2).

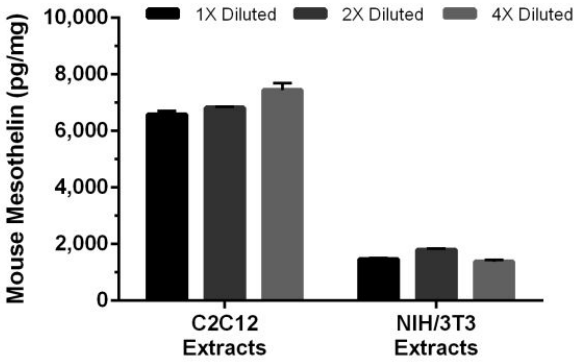


Figure 5. Interpolated concentrations of Mesothelin in mouse cell extract samples. The concentrations of Mesothelin were measured in duplicates, interpolated from the Mesothelin standard curve and corrected for sample dilution. Note that 1X Diluted C2C12 cell extract samples were pre-diluted to 125 μg/mL. Note that 1X Diluted NIH/3T3 cell extract samples were pre-diluted to 250 μg/mL. The interpolated, dilution factor-corrected values are plotted in pg of Mesothelin per mg of extract (mean +/- SD, n=2).

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant mouse Mesothelin protein in serum, plasma (citrate and EDTA), cell culture supernatant and, cell and tissue extract samples only.

Milk and saliva samples have not been tested with this kit.

The kit is not compatible with plasma (heparin) and urine samples.

18. SPECIES REACTIVITY

This kit recognizes mouse Mesothelin protein.

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

Rat

Hamster

Rabbit

Dog

Pig

Human

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)