ab215083 – Human MMP1 SimpleStep ELISA® Kit

For the quantitative measurement of MMP1 in human serum, plasma (citrate), plasma (heparin), cell culture supernatant, and cell extract.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab215083

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pages 6-8.

Storage and Stability: Store kit at 2-8°C 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 Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human MMP1 Capture Antibody 10X	600 µL	+4°C
Human MMP1 Detector Antibody 10X	600 µL	+4°C
Human MMP1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent 50BS	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

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.

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.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

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.

Sample Diluent 50BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue.

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

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.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

For serum and plasma samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of Sample Diluent 50BS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL Sample Diluent 50BS. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 10,000 pg/mL **Standard #1** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 150 µL of Sample Diluent 50BS into numbers 2-8.
4. Use the **Standard #1** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	150	0	10,000	10,000
2	Standard#1	150	150	10,000	5,000
3	Standard#2	150	150	5,000	2,500
4	Standard#3	150	150	2,500	1,250
5	Standard#4	150	150	1,250	625
6	Standard#5	150	150	625	312.5
7	Standard#6	150	150	312.5	156.25
8	Blank Control	0	150	0	0

For cell culture supernatant samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 120 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	180	120	10,000	6,000
2	Standard#1	150	150	6,000	3,000
3	Standard#2	150	150	3,000	1,500
4	Standard#3	150	150	1,500	750
5	Standard#4	150	150	750	375
6	Standard#5	150	150	375	187.5
7	Standard#6	150	150	187.5	93.75
8	Blank Control	0	150	0	0

For cell culture extract samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 120 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	180	120	10,000	6,000
2	Standard#1	150	150	6,000	3,000
3	Standard#2	150	150	3,000	1,500
4	Standard#3	150	150	1,500	750
5	Standard#4	150	150	750	375
6	Standard#5	150	150	375	187.5
7	Standard#6	150	150	187.5	93.75
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤50%
Plasma – Citrate*	≤50%
Plasma – Heparin*	≤50%
Cell Culture Supernatant	Varies by type
SW480 Cell Extract	63 – 500 µg/ml

*Based on spiked sample

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 samples at least 1:2 into Sample Diluent 50BS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:2 into Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (EDTA) samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets 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. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. 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). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 4ºC.

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.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples 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, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
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. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
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.
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.
9. Alternative to 7 – 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.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

Version 5a | 2024-12-12

ab215083 – Human MMP1 SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human MMP1.

The standard protein in this kit is mature full length human MMP1.

Native signal was detected in serum, cell culture supernatant, and cell extract sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), and plasma (heparin) sample types.

Urine, saliva, milk, CSF, and tissue extract samples have not been tested with this kit.

This kit is incompatible with plasma (EDTA) samples.

INTERFERENCE

50 ng/mL of recombinant TIMP-1, TIMP-2, and TIMP-4 were tested for interference with 1,000 pg/mL of recombinant human MMP1. No interference was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g., linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

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			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.055	0.051	0.053
156.25	0.120	0.110	0.115
312.5	0.185	0.173	0.179
625	0.300	0.311	0.306
1,250	0.521	0.532	0.527
2,500	1.003	0.950	0.977
5,000	1.832	1.796	1.814
10,000	3.226	3.109	3.167

Table 1. Example of human MMP1 standard curve in Sample Diluent 50BS. The MMP1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.058	0.060	0.059
93.75	0.123	0.131	0.127
187.5	0.179	0.187	0.183
375	0.291	0.300	0.296
750	0.516	0.519	0.518
1,500	0.929	0.938	0.933
3,000	1.805	1.929	1.867
6,000	3.217	3.253	3.235

Table 2. Example of human MMP1 standard curve in Sample Diluent NS. The MMP1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.061	0.061	0.061
93.75	0.131	0.135	0.133
187.5	0.200	0.205	0.202
375	0.326	0.332	0.329
750	0.580	0.569	0.575
1,500	1.066	1.012	1.039
3,000	1.996	1.859	1.927
6,000	3.377	3.242	3.309

Table 3. Example of human MMP1 standard curve in 1X Cell Extraction Buffer PTR. The MMP1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 50BS	26	41.07 pg/mL
Sample Diluent NS	26	19.64 pg/mL
1X Cell Extraction Buffer PTR	30	22.2 pg/mL

Recovery

Three concentrations of MMP1 were spiked into the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Human Serum	106	98 – 117
50% Human Plasma – Citrate	94	89 – 101
50% Human Plasma – Heparin	95	93 – 100
10% SW480 Supernatant	103	91 – 114
50 µg/mL SW480 Cell Extract	104	96 – 111

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.

Recombinant MMP1 was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	50% Human Plasma (Heparin)
Undiluted	pg/mL	5,979	5,206	5,241
	% Expected value	100	100	100
2	pg/mL	2,891	2,925	2,670
	% Expected value	97	112	102
4	pg/mL	1,435	1,519	1,444
	% Expected value	96	117	110
8	pg/mL	715	654	716
	% Expected value	96	100	109
16	pg/mL	328	339	343
	% Expected value	88	104	105

Native MMP1 was measured in the following biological samples in a 2-fold dilution series. Cell supernatant sample dilutions are made in Sample Diluent NS. Cell extract sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	12.5% SW480 Supernatant	500 µg/mL SW480 Cell Extract
Undiluted	pg/mL	1,590	930.9
	% Expected value	100	100
2	pg/mL	792	471.2
	% Expected value	100	101
4	pg/mL	362	236.0
	% Expected value	91	101
8	pg/mL	184	116.1
	% Expected value	93	100
16	pg/mL	100	ND
	% Expected value	101	ND

ND – Not Detected – below product dynamic range

Precision

Mean coefficient of variations of interpolated values of MMP1 from three concentrations of SW480 supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	4.0	2.8

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human MMP1 Capture Antibody 10X	600 µL	+4°C
Human MMP1 Detector Antibody 10X	600 µL	+4°C
Human MMP1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	50 mL	+4°C
Cell Extraction Enhancer Solution 50X	5 mL	+4°C
Sample Diluent 50BS	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 wells	+4°C
Plate Seal	1	+4°C

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 in a 384-well plate.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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.

Sample Diluent 50BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 250 mL 1X Cell Extraction Buffer PTR combine 195 mL deionized water, 50 mL Cell Extraction Buffer PTR 5X and 5 mL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 200 mL 1X Wash Buffer PT combine 20 mL Wash Buffer PT 10X with 180 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CPI2. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

For serum and plasma samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of Sample Diluent 50BS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL Sample Diluent 50BS. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 10,000 pg/mL **Standard #1** Solution.
2. Label seven tubes, Standards 1– 7.
3. Add 75 µL of Sample Diluent 50BS into numbers 2-7.
4. Use the **Standard #1** to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	75	0	10,000	10,000
2	Standard#1	75	75	10,000	5,000
3	Standard#2	75	75	5,000	2,500
4	Standard#3	75	75	2,500	1,250
5	Standard#4	75	75	1,250	625
6	Standard#5	75	75	625	312.5
7	Blank Control	0	75	0	0

Note: The 384 well curve uses 6 points, compared to the 7-point 96 well curve.

For cell culture supernatant samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 60 µL of Sample Diluent NS into tube number 1 and 75 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	90	60	10,000	6,000
2	Standard#1	75	75	6,000	3,000
3	Standard#2	75	75	3,000	1,500
4	Standard#3	75	75	1,500	750
5	Standard#4	75	75	750	375
6	Standard#5	75	75	375	187.5
7	Standard#6	75	75	187.5	93.75
8	Blank Control	0	75	0	0

For cell culture extract samples, follow these instructions:

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the MMP1 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MMP1 standard by adding 500 µL 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 60 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 75 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	90	60	10,000	6,000
2	Standard#1	75	75	6,000	3,000
3	Standard#2	75	75	3,000	1,500
4	Standard#3	75	75	1,500	750
5	Standard#4	75	75	750	375
6	Standard#5	75	75	375	187.5
7	Standard#6	75	75	187.5	93.75
8	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
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.

Assay Procedure for 384-well Plate Format

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 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.
7. Add 25 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. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: 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 25 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

Version 5a | 2024-12-12