

Research Use Only. Not for use in diagnostic procedures

## Human matrix metalloproteinase (hMMP-9) AlphaLISA Detection Kit

Product number: AL3138

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### Product Information

**Application:** This kit is designed for the quantitative determination of human MMP-9 in culture medias, cell lysates, human serum, human plasma, human cerebrospinal fluid and

human saliva using a homogeneous AlphaLISA assay (no wash steps). The assay shows no significant cross reactivity with hMMP-1, hMMP-2, hMMP-3, hMMP-7, hMMP-8, hMMP-10, hMMP-12, hMMP-13, and hMMP-23.

Specifications described below are based on assay performed in AlphaLISA Immunoassay Buffer 1X.

**Sensitivity:** Lower Detection Limit (LDL): 3 pg/mL  
Lower Limit of Quantification (LLOQ): 11 pg/mL  
EC<sub>50</sub>: 40 ng/mL

**Dynamic range:** 3 – 1 000 000 pg/mL

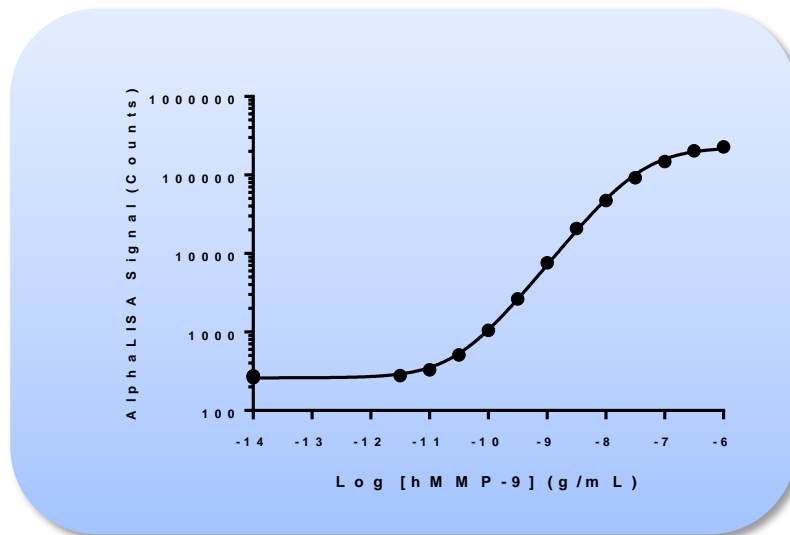


Figure 1. Typical sensitivity curve in AlphaLISA Immunoassay Buffer. The data was generated using a white Optiplate™-384 microplate and the EnVision® Multilabel Plate Reader 2102 with Alpha option.

**Storage:** Store kit in the dark at +4 °C.

**Stability:** This kit is stable for at least 6 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

### Quality Control

Lot to lot consistency is confirmed in an AlphaLISA assay. Maximum and minimum signals, EC<sub>50</sub> and LDL were measured on the EnVision Multilabel Plate Reader with Alpha option using the protocol described in this technical data sheet. We certify that these results meet our quality release criteria. Maximum counts may vary between bead lots and the instrument used, with no impact on LDL measurement.

EC<sub>50</sub>: ng/mL  
LDL: pg/mL  
LLOQ: pg/mL

## Analyte of Interest

Human MMP-9 is a 92 kDa protein, a member of the matrix metalloproteinase family. It is also known as gelatinase B. Its primary function is cleavage of proteins of the extracellular matrix, including fibronectin and collagen IV and V. MMP-9 is involved in many biological pathways such as development, reproduction angiogenesis, wound repair and immune response. It is also involved in pathologies such as cancer, arthritis and cardiovascular diseases.

## Description of the AlphaLISA Assay

AlphaLISA technology allows the detection of molecules of interest in buffer, cell culture media, serum and plasma in a highly sensitive, quantitative, reproducible and user-friendly mode. In an AlphaLISA assay, a biotinylated anti-analyte antibody binds to the Streptavidin-coated Alpha Donor beads, while another anti-analyte antibody is conjugated to AlphaLISA acceptor beads. In the presence of the analyte, the beads come into close proximity. The excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 2).

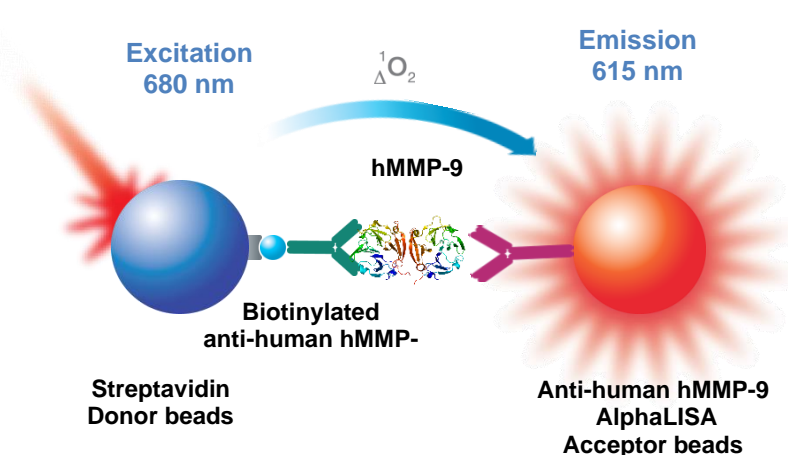


Figure 2. AlphaLISA Assay Principle.

## Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- Take precautionary measures to avoid contamination of the reagent solutions.
- The Biotinylated Anti-Analyte Antibody contains sodium azide. Contact with skin or inhalation should be avoided.

## Kit Content: Reagents and Materials

Kit components	AL3138HV (100 assay points) **	AL3138C (500 assay points)	AL3138F (5000 assay points)
AlphaLISA Anti-hMMP-9 Acceptor beads stored in PBS, 0.05% Kathon CG/ICP, pH 7.2	20 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	50 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)	500 µL @ 5 mg/mL (1 brown tube, <u>white</u> cap)
Streptavidin (SA)-coated Donor beads stored in 25 mM HEPES, 100 mM NaCl, 0.05% Kathon CG/ICP, pH 7.4	40 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	200 µL @ 5 mg/mL (1 brown tube, <u>black</u> cap)	1 000 µL @ 5 mg/mL (2 brown tubes, <u>black</u> cap)
Biotinylated Anti-hMMP-9 Antibody stored in PBS, 0.1% Tween-20, 0.05% NaN <sub>3</sub> , pH 7.4	20 µL @ 500 nM (1 tube, <u>black</u> cap)	50 µL @ 500 nM (1 tube, <u>black</u> cap)	500 µL @ 500 nM (1 tube, <u>black</u> cap)
Lyophilized hMMP-9 ***	1 µg (1 tube, <u>clear</u> cap)	1 µg (1 tube, <u>clear</u> cap)	1 µg (1 tube, <u>clear</u> cap)
AlphaLISA Immunoassay Buffer (10X) *	2 mL, 1 small bottle	10 mL, 1 medium bottle	100 mL, 1 large bottle

\* Extra buffer can be ordered separately (cat # AL000C: 10 mL, cat # AL000F: 100 mL).

\*\* The number of assay points is based on an assay volume of 100 µL in 96-well plates or 50 µL in 96- or 384-well assay plates using the kit components at the recommended concentrations.

\*\*\* Reconstitute lyophilized analyte in 100 µL Milli-Q® grade H<sub>2</sub>O. The reconstituted analyte should be used within 60 minutes or aliquoted into screw-capped 0.5 mL polypropylene vials and stored at -20 °C for future experiments. The aliquoted analyte stored at -20 °C is stable up to 30 days. The reconstituted material can tolerate up to three freeze-thaw cycles. However, freeze-thaw cycles should be avoided if possible. One vial contains an amount of analyte sufficient for performing 10 standard curves. Additional vials can be ordered separately (cat # AL3138S).

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal. Note that sodium azide from the Biotinylated Antibody stock solution will not interfere with the AlphaLISA signal (0.0001% final in the assay).

### Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Plus Adhesive Sealing Film	PerkinElmer Inc.	6050185
EnVision®-Alpha Reader	PerkinElmer Inc.	-

## Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend all reagents by vortexing before use.
- Use Milli-Q® grade H<sub>2</sub>O (18 MΩ•cm) to dilute 10X AlphaLISA Immunoassay Buffer.
- When diluting the standard or samples, change tips between each standard or sample dilution. When loading reagents in the assay microplate, change tips between each standard or sample addition and after each set of reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Plus Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.
- The standard curves shown in this technical data sheet are provided for information only. A standard curve must be generated for each experiment.

## Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The protocol described below is an example for generating one standard curve in a 50 µL final assay volume (48 wells, triplicate determinations). The protocols also include testing samples in 452 wells. If a different number of samples are tested, the volumes of all reagents have to be adjusted accordingly, as shown in the table below. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes.
- The standard dilution protocol is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.
- Use of four background points in triplicate (12 wells) is recommended when LDL/LLOQ is calculated. One background point in triplicate (3 wells) can be used when LDL/LLOQ is not calculated.

Format	# of data points	Volume				Plate recommendation
		Final	Sample	AlphaLISA Acceptor beads and Biotinylated Antibody	SA-Donor beads	
AL3138HV	100	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
AL3138C	250	100 µL	10 µL	10 µL	80 µL	White OptiPlate-96 (cat # 6005290) White ½ AreaPlate-96 (cat # 6005560)
	500	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate™-384 (cat # 6005350)
	1 250	20 µL	2 µL	2 µL	16 µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate™-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	2 500	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)
AL3138F	5 000	50 µL	5 µL	5 µL	40 µL	White ½ AreaPlate-96 (cat # 6005560) White OptiPlate-384 (cat # 6007290) Light gray AlphaPlate-384 (cat # 6005350)
	12 500	20 µL	2 µL	2 µL	16µL	Light gray AlphaPlate-384 (cat # 6005350) ProxiPlate-384 Plus (cat # 6008280) White OptiPlate-384 (cat # 6007290)
	25 000	10 µL	1 µL	1 µL	8 µL	Light gray AlphaPlate-1536 (cat # 6004350)

**2-Step High concentration Protocol described below is for 500 assay points including one standard curve (48 wells) and samples (452 wells). If a different number of samples are tested, the volumes of all reagents have to be adjusted accordingly.**

- 1) Preparation of 1X AlphaLISA Immunoassay Buffer:
  - a. Add 5 mL of 10X AlphaLISA Immunoassay Buffer to 45 mL Milli-Q grade water.
- 2) Preparation human MMP-9 analyte standard dilutions:
  - a. Reconstitute analyte by adding 100  $\mu$ L of Milli-Q grade water to one tube of analyte to generate a 10  $\mu$ g/mL solution.
  - b. Prepare standard dilutions as follows in 1X AlphaLISA Immunoassay Buffer

Tube	Vol. of human MMP-9 ( $\mu$ L)	Vol. of diluent ( $\mu$ L) *	[human MMP-9] in standard curve	
			(g/mL in 5 $\mu$ L)	(pg/mL in 5 $\mu$ L)
A	10 $\mu$ L of human MMP-9 stock	90	1.00E-06	1 000 000
B	60 $\mu$ L of tube A	140	3.00E-07	300 000
C	60 $\mu$ L of tube B	120	1.00E-07	100 000
D	60 $\mu$ L of tube C	140	3.00E-08	30 000
E	60 $\mu$ L of tube D	120	1.00E-08	10 000
F	60 $\mu$ L of tube E	140	3.00E-09	3 000
G	60 $\mu$ L of tube F	120	1.00E-09	1 000
H	60 $\mu$ L of tube G	140	3.00E-10	300
I	60 $\mu$ L of tube H	120	1.00E-10	100
J	60 $\mu$ L of tube I	140	3.00E-11	30
K	60 $\mu$ L of tube J	120	1.00E-11	10
L	60 $\mu$ L of tube K	140	3.00E-12	3
M ** (background)	0	100	0	0
N ** (background)	0	100	0	0
O ** (background)	0	100	0	0
P ** (background)	0	100	0	0

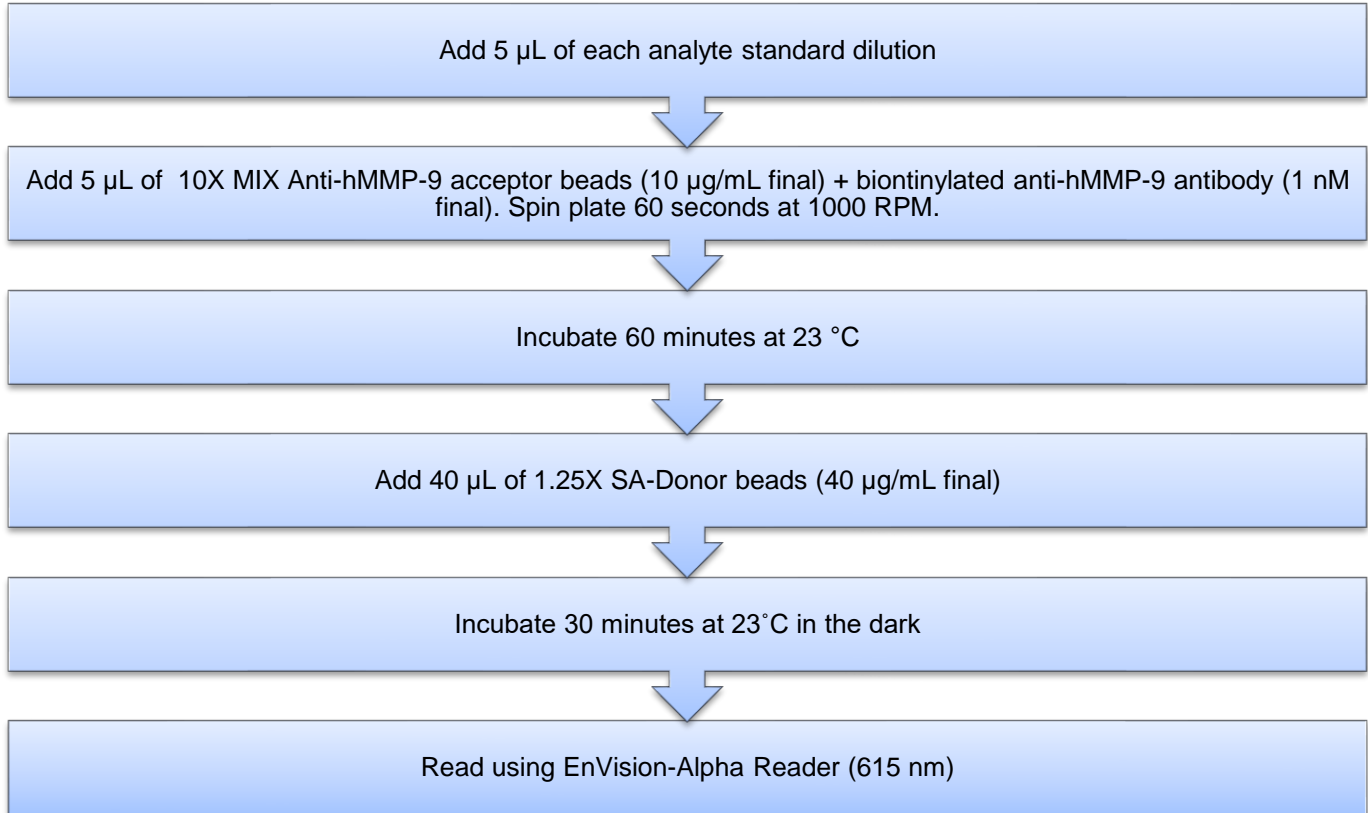
\* Dilute standards in diluent (e.g. 1X AlphaLISA Immunoassay Buffer).  
At low concentrations of analyte, a significant amount of analyte can bind to the vial. Therefore, load the analyte standard dilutions in the assay microplate within 60 minutes of preparation.

\*\* Four background points in triplicate (12 wells) are used when LDL is calculated. If LDL does not need to be calculated, one background point in triplicate can be used (3 wells).

- 3) Preparation of 10X MIX AlphaLISA Anti-hMMP-9 Antibody Acceptor beads (100  $\mu$ g/mL) + Biotinylated Anti-hMMP-9 Antibody (10 nM):
  - a. Prepare just before use.
  - b. Add 50  $\mu$ L of 5 mg/mL AlphaLISA Anti-hMMP-9 antibody Acceptor Bead and 50  $\mu$ L of 500 nM biotinylated anti-hMMP-9 Antibody to 2 400  $\mu$ L of 1X AlphaLISA Immunoassay Buffer.
- 4) Preparation of 1.25X Streptavidin (SA) Donor beads (50  $\mu$ g/mL):
  - a. Prepare just before use.

- b. Keep the beads under subdued laboratory lighting.
- c. Add 200  $\mu\text{L}$  of 5 mg/mL SA-Donor beads to 19 800  $\mu\text{L}$  of 1X AlphaLISA Immunoassay Buffer.

5) In a white Optiplate (384 wells):



## Data Analysis

- Calculate the average count value for the background wells.
- Generate a standard curve by plotting the AlphaLISA counts versus the concentration of analyte. A log scale can be used for either or both axes. No additional data transformation is required.
- Analyze data according to a nonlinear regression using the 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a  $1/Y^2$  data weighting (the values at maximal concentrations of analyte after the hook point should be removed for correct analysis).
- The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.
- The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve. Alternatively, the true LLOQ can be determined by spiking known concentrations of analyte in the matrix and measuring the percent recovery, and then determining the minimal amount of spiked analyte that can be quantified within a given limit (usually +/- 20% or 30% of the real concentration).
- Read from the standard curve the concentration of analyte contained in the samples.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



## Assay Performance Characteristics

AlphaLISA assay performance described below was determined using the 2-step protocol using AlphaLISA Immunoassay Buffer (IAB).

- Assay Sensitivity:

The LDL was calculated as described above. The values correspond to the lowest concentration of analyte that can be detected in a volume of 5  $\mu$ L using the recommended assay conditions.

LDL (pg/mL)	Buffer *	# of experiments
3	IAB	7
9.4	AlphaLISA Lysis Buffer (ALB)	6
10.8	DMEM	6
11.5	DMEM + 10% FBS	6
35.7	RPMI	6
20.6	RPMI + 10% FBS	6
33.4	FBS	6
11.8	10% FBS in IAB	6

\* The standard was prepared in these diluents and all other components were diluted in IAB. Note that LDL can be decreased (i.e. sensitivity increased) by increasing the volume of analyte in the assay (e.g. use 10  $\mu$ L of analyte in a final assay volume of 50  $\mu$ L).

Performing assays in RPMI 1640 will see some interference from the media. Users should check if expected levels of hMMP-9 to be detected in the target cell supernatant are above the LDL of hMMP-9 in RPMI.

- Assay Precision:

The following assay precision data were calculated from the three independent assays using two different kit lots. In each lot, the analytes were prepared in IAB, AlphaLISA Lysis Buffer, DMEM (with or without 10% FBS), RPMI (with or without 10% FBS), FBS or 10% FBS. All other components were prepared in IAB. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format.

- Intra-assay precision:

The intra-assay precision was determined by averaging 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

human MMP-9	IAB	Lysis Buffer	DMEM	DMEM + 10% FBS	RPMI	RPMI + 10% FBS	FBS	10% FBS in IAB
<b>CV (%)</b>	6.3	4.1	5.0	3.2	4.9	3.8	7.7	4.3

- Inter-assay precision:

The inter-assay precision was determined comparing 6 experiments each with 12 independent determinations in triplicate. Shown as CV%.

human MMP-9	IAB	Lysis Buffer	DMEM	DMEM + 10% FBS	RPMI	RPMI + 10% FBS	FBS	10% FBS in IAB
<b>CV (%)</b>	6.4	9.4	7.2	10.2	11.3	12.4	9.2	9.4

- Spike Recovery:

Known concentrations of analyte were spiked into IAB, AlphaLISA lysis buffer, DMEM (with or without 10% FBS), RPMI (with or without 10% FBS), FBS and 10% FBS in IAB or. All samples, including non-spiked buffer were measured in the assay. Note that the standard curves were prepared in the matrices used for the samples. All other components were diluted in IAB.

Spiked human MMP-9 (ng/mL)	% Recovery							
	IAB	Lysis Buffer	DMEM	DMEM + 10% FBS	RPMI	RPMI + 10% FBS	FBS	10% FBS in IAB
300	92	97	103	112	84	95	81	105
30	85	120	110	88	78	102	89	109
3	88	122	95	93	82	119	77	112

## Human matrices testing

The following matrices were used to test for the presence of hMMP-9

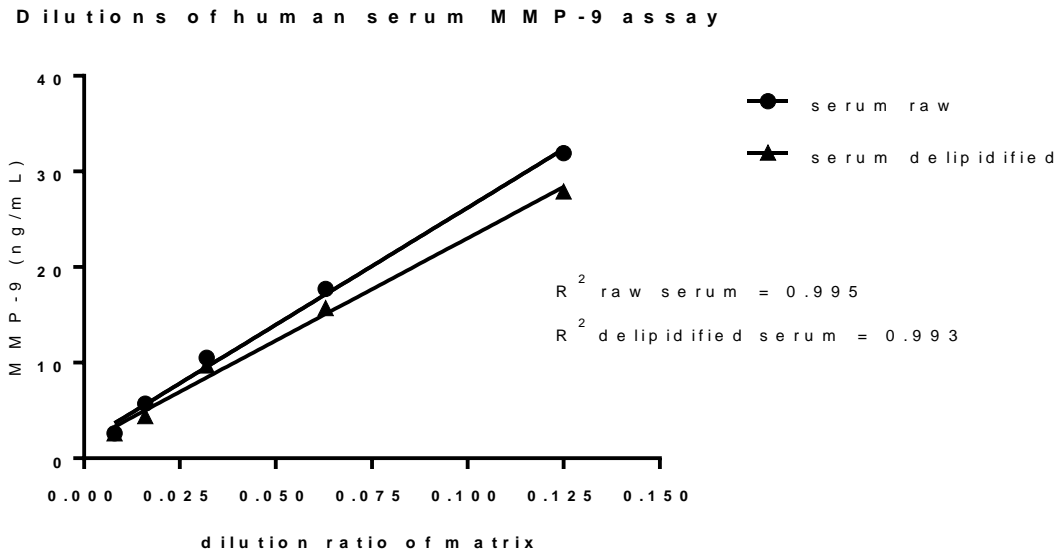
- Human serum\*
- Human plasma\*
- HeLa cell lysate (commercial product from Millipore 47-205).

\* Samples of human serum and plasma were also tested by removing excess lipids by centrifugation.

All samples except for cell lysate are from pooled healthy donors.

All matrices were used raw or diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 or 1:128 in IAB and then tested using the protocol as described above. All sample results were plotted on a standard curve of hMMP-9 in AlphaLISA immunoassay buffer.

### Human serum



A m o u n t i n t o t a l r a w s e r u m = 2 5 5 n g / m L

A m o u n t i n d e l i p i d i f i e d s e r u m = 2 2 4 n g / m L

A dilution of the sample 1:8 in immunoassay buffer was necessary to offer linear dilution curves.

A spike and recovery experiment was performed on the material after a 1:10 dilution.

A sample of human serum was diluted 1:10 in AlphaLISA Immunoassay buffer. Then, 100, 10 and 1 ng/mL of recombinant human MMP-9 was added to the samples. The samples were tested using the hMMP-9 detection assay and plotted against a standard curve of hMMP-9 in 10% FBS in IAB.

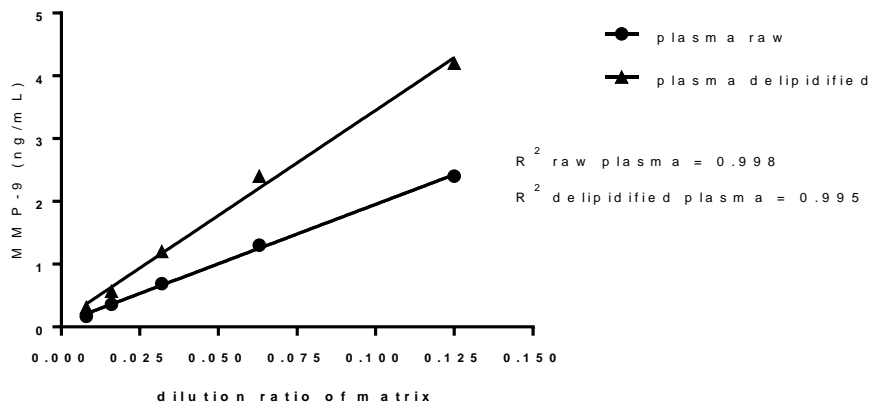
Spiked amounts (ng/mL)	[hMMP-9] (ng/mL) (raw)	[hMMP9] (ng/mL) (corrected)	% recovery
none	28.1	28.1	NA

100	140.3	112.2	112
10	36.6	8.5	85
1	29.3	1.2	120

The results in the corrected column correspond to the observed data minus the amount present in the unspiked sample.

## Human plasma

Dilutions of human plasma MMP-9 assay



Amount in total raw plasma = 19.2 ng/mL

Amount in delipidified plasma = 33.6 ng/mL

Plasma samples were very cloudy. Lipids were removed by centrifugation. This step is recommended if samples look cloudy or have lipids agglomerates present.

A dilution of the sample 1:8 in immunoassay buffer was necessary to offer linear dilution curves.

A spike and recovery experiment was performed on the material after a 1:10 dilution.

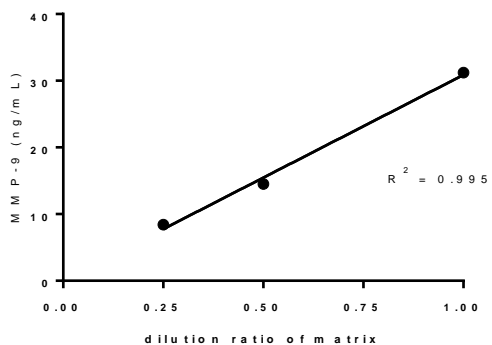
A sample of human plasma was diluted 1:10 in AlphaLISA Immunoassay buffer. Then, 100, 10 and 1 ng/mL of recombinant human MMP-9 was added to the samples. The samples were tested using the hMMP-9 detection assay and plotted against a standard curve of hMMP-9 in 10% FBS in IAB.

Spiked amounts (ng/mL)	[hMMP-9] (ng/mL) (raw)	[hMMP9] (ng/mL) (corrected)	% recovery
none	2.25	2.25	NA
100	93.5	91.25	91.3
10	14.5	12.25	123
1	3.38	1.13	113

The results in the corrected column correspond to the observed data minus the amount present in the unspiked sample.

## Human HeLa cell lysate

Dilutions of human HeLa cells extract MMP-9 assay



Amount in raw HeLa extract = 31.2 pg/mL

The protein is detectable even at basal level.

## Specificity Experiments

Cross-reactivity of the human MMP-9 kit was tested using the following proteins up to 1 µg/mL in IAB.

Protein	% Cross-reactivity
hMMP-1	0
hMMP-2	0
hMMP-3	0
hMMP-7	0
hMMP-8	< 0.01
hMMP-10	0
hMMP-12	0
hMMP-13	0
hMMP-23	0.1
Mouse MMP-9	0.3

## Troubleshooting Guide

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at:

[http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha\\_troubleshoot.xhtml](http://www.perkinelmer.com/in/resources/technicalresources/applicationsupportknowledgebase/alphalisa-alphascreen-no-washassays/alpha_troubleshoot.xhtml)

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