BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human MRP14

Cat. No. 445307 1 Plate

445308 5 Plates

ELISA Kit for Accurate Quantitation of Human MRP14 from Serum and Plasma

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

Migration inhibitory factor-related protein 14 (MRP14), also known as S100 Calcium-Binding Protein A9 (S100A9) and calgranulin B, is a 14 kD member of the S100 family, which is a group of calcium-binding proteins that regulate intra- and extracellular activities of other effector proteins, enzymes, receptors, cytoskeletal subunits, and nucleic acids. MRP14 consists of 114 amino acids and is expressed in granulocytes, monocytes, macrophages, and tumor cells. This protein predominantly forms the heterodimers (known as calprotectin) and heterotetramers with MRP8 (S100A8), but also forms a homodimer.

Human MRP14 shares significant amino acid sequence identity with MRP14 from other species, including >79%, 72%, 70%, 68%, 64%, and 57% from non-human primates, equine, bovine, porcine, rat, and mouse, respectively.

MRP14 has major roles as an inflammatory mediator. It regulates myeloid cell function by binding to Toll-like receptor-4 and the receptor for advanced glycation end-products (RAGE). MRP14 induces neutrophil chemotaxis and adhesion, promotes phagocytosis via activation of SYK, PI3K/AKT, and ERK1/2, and induces degranulation of neutrophils by a MAPK-dependent mechanism. MRP14 is a potential biomarker for many diseases as it plays important roles in inflammatory disorders and autoimmune diseases, and it has been implicated with carcinogenesis and acute myocardial infarction.

The LEGEND MAX™ Human MRP14 Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human MRP14 capture antibody. The Detection Antibody is a biotinylated form of the same mouse monoclonal anti-human MRP14 antibody. This kit has been analytically validated to accurately measure MRP14 homodimer in serum and plasma samples with ready-to-use reagents.

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Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-human MRP14 Pre-coated 96-well Strip Microplate	1 plate	5 plates		76312
Human MRP14 Detection Antibody	1 bottle	5 bottles	12 mL	76313
Human MRP14 Standard	1 vial	5 vials	lyophilized	76315
Matrix B	1 vial	5 vials	lyophilized	77587
Avidin-HRP	1 bottle	5 bottles	12 mL	77897
Assay Buffer B	1 bottle	5 bottles	25 mL	79128
Assay Diluent E	1 bottle	5 bottles	25 mL	77589
Wash Buffer (20X)	1 bottle	5 bottles	50 mL	78233
Substrate Solution F	1 bottle	5 bottles	12 mL	76335
Stop Solution	1 bottle	5 bottles	12 mL	79133
Plate Sealers	4 sheets	20 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate Strips</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td rowspan="2">The remaining reconstituted standard or matrix stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Matrix B</td></tr>
<tr><td>Detection Antibody</td><td rowspan="7">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP</td></tr>
<tr><td>Assay Buffer B</td></tr>
<tr><td>Assay Diluent E</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

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Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 20 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in containing tubes containing heparin, citrate, or EDTA, and ensure a clean stick with no hemolysis. Centrifuge immediately for 20 minutes at 1,000 x *g*. Carefully remove supernatant and assay immediately or store at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Matrix B by adding 5 mL of Assay Buffer B to the vial. Allow the reconstituted matrix to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. Reconstitute the lyophilized Human MRP14 Standard by adding the volume of Assay Buffer B to make a 100 ng/mL standard stock solution (refer to LEGEND MAX™ Kit Lot-Specific Certificate of Analysis/LEGEND MAX™ Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
4. For measuring serum and plasma samples, a 2-fold dilution using Assay Buffer B is necessary to ensure assay that the measurement is accurate. For example, dilute 100 µL of sample in 100 µL of Assay Buffer B.

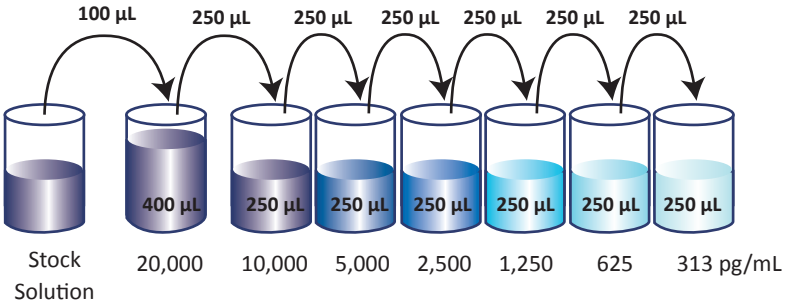
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 20,000 pg/mL top standard by diluting 100 µL of the standard stock solution in 400 µL of Assay Diluent E. Perform six two-fold serial dilutions of the 20,000 pg/mL top standard in separate tubes using Assay Diluent E as the diluent. Thus, the human MRP14 standard concentrations in the tubes are 20,000 pg/mL, 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 313 pg/mL, respectively. Assay Diluent E serves as the zero standard (0 pg/mL).



4. Add 50 µL Matrix B to each well that will contain standards.

5. Add 50 µL Assay Diluent E to each well that will contain samples.

6. Add 50 µL of standard dilutions or properly diluted samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer. Wash the plate with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.

9. Add 100 µL of Human MRP14 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 8.

11. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 8. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

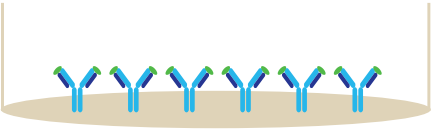
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 minutes in the dark. Wells containing human MRP14 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

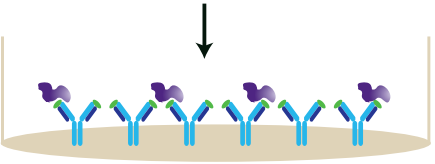
15. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

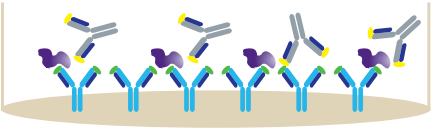
1. Add 50 µL Matrix B to wells that will contain standards
   Add 50 µL Assay Diluent E to wells that will contain samples



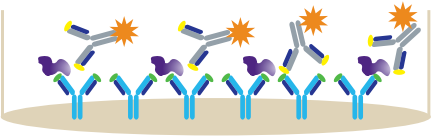
2. Add 50 µL diluted standards or samples
   Incubate 2 hrs, RT, shaking



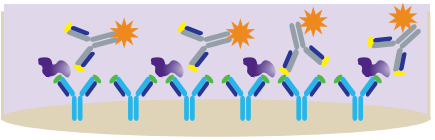
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



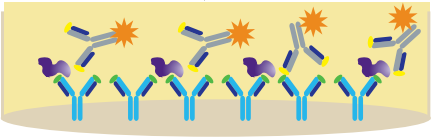
4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 mins, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 15 mins, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

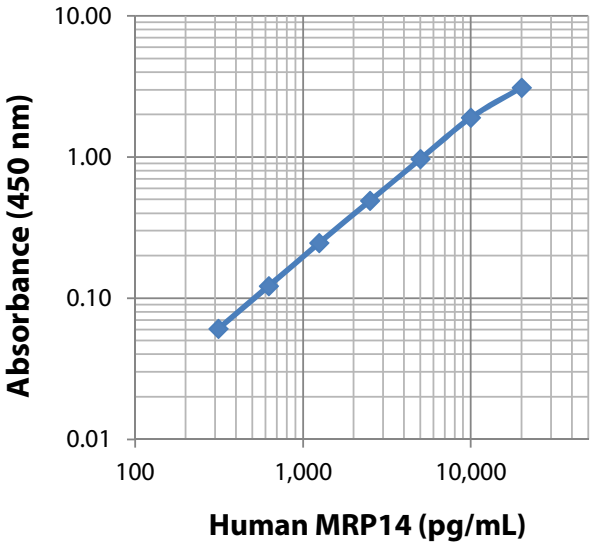
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the protein concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at 50 ng/mL except where the concentration is specified.

Human	MRP8 (100 ng/mL), S100A12, IL-1α, IL-1β, IL-1R, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, IL-15, IL-17A, IL-17F, IL-21, IL-27, IL-36α, IL-36γ, TGF-α, TNF-α, IFN-γ, CCL1

This kit has ~1.6% reactivity with human MRP8/14 recombinant heterodimer (Calprotectin). However, this is not true for natural samples, as 33% of tested serum samples do not show detectable levels of MRP14 despite an average reported concentration of 1.24 ug/mL of MRP8/14 heterodimer in serum samples.

Sensitivity: The minimum detectable concentration is 63.1 pg/mL ± 34.6 pg/mL (mean ± 2SD; n=9).

Linearity: Human samples were spiked with recombinant human MRP14 and diluted with Assay Buffer B to produce samples with values within the dynamic range, and then assayed with the kit to determine the dilutional linearity up to 8-fold dilutions.

Sample Type	N	% Linearity
Serum	5	109
Heparin Plasma	5	107
EDTA Plasma	5	104
Citrate Plasma	5	85

Recovery: Recombinant human MRP14, at concentrations of 10,000 pg/mL, 2,500 pg/mL, and 625 pg/mL, was spiked into the following human samples. **Samples were diluted prior to spiking.*

Sample Type	N	% Recovery
Serum*	5	108
Heparin Plasma*	5	98
EDTA Plasma*	10	100
Citrate Plasma*	5	102

Intra-Assay Precision: Two samples with different concentrations of human serum were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	6,068	870
Standard Deviation	370	40
% CV	6.1	4.6

Inter-Assay Precision: Two different concentrations of human serum were assayed in four independent assays by four different users.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	5,246	787
Standard Deviation	765	99
% CV	14.6	12.5

Biological Samples

Serum and Plasma: Matched serum and plasma samples from 15 donors were tested in one assay.

Sample Type	N	% Detectable	Min. (pg/mL)	Max. (pg/mL)	Median (pg/mL)
Serum	15	67	ND*	8,125	2,635
Heparin Plasma	15	47	ND*	7,567	2,950
EDTA Plasma	15	80	ND*	7,036	1,151
Citrate Plasma	15	33	ND*	6,160	2,787

**ND = Not Detectable*

Cell Culture Supernatant: Cell culture supernatant from the following unstimulated cell lines were tested for natural MRP14. No MRP14 was detected.

Cell Line	Type	Time	Growth Media
PBMC	Peripheral Blood Mononuclear Cell	Day 4	DMEM + 10% FBS
A431	Epidermoid Carcinoma	Day 4	RPMI + 10% FBS
A549	Alveolar Basal Epithelial Adenocarcinoma	Day 4	RPMI + 10% FBS
HaCat	Aneuploid Immortal Keratinocyte	Day 4	DMEM + 10% FBS
HepG2	Hepatocellular Carcinoma	Day 4	EMEM + 10% FBS
HT29	Colon Adenocarcinoma	Day 4	DMEM + 10% FBS
U87MG	Glioblastoma	Day 4	EMEM + 10% FBS

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Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes

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Notes

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LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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