PRODUCT INFORMATION & MANUAL

Human S100A8/S100A9 Heterodimer Valukine™ ELISA

Catalog Number: VAL248

For the quantitative determination of natural and recombinant human S100A8/S100A9 Heterodimer concentrations

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

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

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

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

S100A8 (also known as MRP8, Calgranulin A, and CP-10) and S100A9 (also known as MRP14 and Calgranulin B) are pro-inflammatory members of the S100 family of secreted calcium binding proteins (1, 2). The 10 kDa human S100A8 and 14 kDa S100A9 each contain two EF-hand calcium binding motifs. Human S100A8 shares 57% and 61% amino acid (aa) sequence identity with mouse and rat S100A8, respectively. Human S100A9 shares 57% and 62% amino acid sequence identity with mouse and rat S100A9, respectively (3, 4). In the presence of calcium or zinc, S100A8 and S100A9 can associate into disulfide-linked homodimers and 34-35 kDa heterodimers known as S100A8/S100A9 Heterodimer or Calprotectin (5-8).

S100A8 and S100A9 are upregulated in neutrophils, monocytes, Schwann cells, and keratinocytes at sites of inflammation (9-15). The S100A8/S100A9 Heterodimer is elevated in rheumatoid arthritis synovial fluid and in the serum of cardiovascular disease, atopic dermatitis, and psoriatic arthritis patients (1, 11, 15-17). Its levels correlate with body adiposity and leukocyte count (18). S100A8/S100A9 Heterodimer exerts its effects through the receptors RAGE and TLR4 (13, 19). The heterodimer promotes neutrophil infiltration into sites of inflammation and inflammatory cytokine production by monocytes (6, 10, 11, 14, 20). It promotes bone spur formation in osteoarthritis (21), upregulation of Complement Component C3 (20), astrocyte proliferation (19), and the suppression of tumor growth by promoting the influx and activation of NK cells (12, 13). The S100A8/S100A9 Heterodimer additionally binds to fatty acids such as arachidonic acid (9), and it sequesters manganese, thereby restricting the growth of Mn-dependent bacteria (22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human S100A8/S100A9 Heterodimer has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human S100A8/S100A9 Heterodimer present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human S100A8/S100A9 Heterodimer is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human S100A8/S100A9 Heterodimer bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernates, tissue lysates, human serum, human plasma, human saliva, human urine, human milk, and human fecal extract.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent RD5-10 and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

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

Inter-assay Precision (Precision between assays)

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

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (ng/mL)	5.64	12.1	25.3	6.09	12.6	24.6
Standard Deviation	0.155	0.370	1.14	0.352	0.606	0.792
CV%	2.7	3.1	4.5	5.8	4.8	3.2

B. RECOVERY

The recovery of human S100A8/S100A9 Heterodimer spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	85	79-96
Lysis buffer* (n=3)	94	80-104
Human Urine* (n=4)	100	91-113
Human Milk* (n=4)	96	83-103

*Samples were diluted prior to assay.

C. SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human S100A8/S100A9 Heterodimer ranged from 0.005-0.215 ng/mL. The mean MDD was 0.086 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human S100A8/S100A9 Heterodimer produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human S100A8/S100A9 Heterodimer and diluted with Calibrator Diluent RD5-10 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates* (n=4)	Lysis buffer (n=3)	Human Serum* (n=4)	Human EDTA plasma* (n=4)	Human Heparin plasma* (n=4)
1:2	Average % of Expected	104	107	104	106	104
	Range (%)	100-112	99-119	99-109	102-109	101-109
1:4	Average % of Expected	104	110	110	111	110
	Range (%)	99-112	100-120	106-116	102-115	99-119
1:8	Average % of Expected	103	112	111	111	116
	Range (%)	101-105	105-121	107-118	100-117	101-124
1:16	Average % of Expected	99	114	113	104	115
	Range (%)	95-104	103-125	106-116	101-109	101-124

Dilution		Human Saliva* (n=4)	Human Urine* (n=4)	Human Milk* (n=4)	Human Fecal extract* (n=3)
1:2	Average % of Expected	103	102	103	103
	Range (%)	100-107	101-105	101-107	96-112
1:4	Average % of Expected	105	104	104	103
	Range (%)	102-115	101-109	99-108	94-113
1:8	Average % of Expected	105	105	102	105
	Range (%)	101-115	97-118	99-105	94-111
1:16	Average % of Expected	101	102	100	88
	Range (%)	92-112	87-118	90-105	86-90

* Samples were diluted prior to assay.

F. SAMPLE VALUES

Human Serum/Plasma/Saliva/Urine/Milk - Samples from apparently healthy volunteers were evaluated for the presence of human S100A8/S100A9 Heterodimer in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Human Serum (n=72)	2015	481-6540	1219
Human EDTA plasma (n=40)	473	127-1395	295
Human Heparin plasma (n=40)	830	298-1640	314
Human Saliva (n=10)	7271	2137-18,960	5336

Human Urine (n=10)	102	0.858-359	99.7
Human Milk (n=10)	1308	34.0-5720	1876

Cell Culture Supernates:

Human peripheral blood leukocytes (PBL; 1×10^6) were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 or 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human S100A8/S100A9 Heterodimer.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	399	575
Stimulated	588	1091

THP-1 human acute monocytic leukemia cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Cells were cultured unstimulated or stimulated with 200 nM PMA until confluent. An aliquot of the cell culture supernate was removed, assayed for human S100A8/S100A9 Heterodimer, and measured 11.6 ng/mL and 38.0 ng/mL, respectively.

Tissue Lysates - Human colon tissue samples were prepared in RIPA buffer with protease inhibitors. The working buffer was kept on ice and used within 1 day. Aliquots of the tissue lysates were removed and assayed for human S100A8/S100A9 Heterodimer.

Sample Type	(ng/mL)
Human colon, normal	14,700
Human colon, cancer	11,215

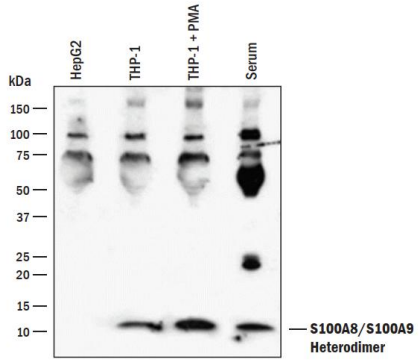
Human Fecal Extracts - Three human fecal samples were extracted by adding 5 mL extraction buffer (0.1 M Tris, 0.015 M NaCl, 1.0 M Urea, 1.0 mM $CaCl_2$, 0.1 M Citric Acid Monohydrate, 5 mg/mL BSA, and 0.25% Gentamycin Sulfate at pH 8.0) into 100 mg sample. Samples were vortexed for 10 minutes and filtered through a 0.2 µm filter. Samples were assayed for human S100A8/S100A9 Heterodimer and measured 81.2 ng/mL, 8.71 ng/mL, and 1175 ng/mL.

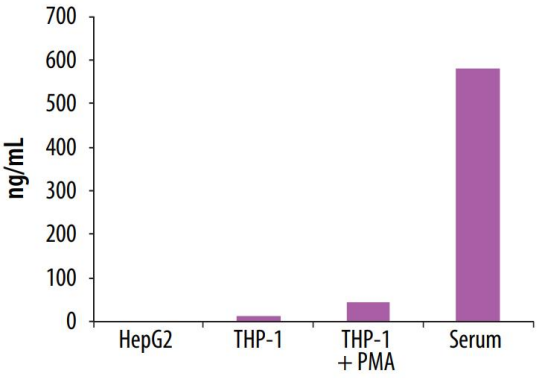
G. SPECIFICITY

This assay recognizes natural and recombinant human S100A8/S100A9 Heterodimer.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent RD5-10 and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human S100A8/S100A9 Heterodimer control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Recombinant mouse:**
RAGE	S100A8
S100A1	S100A9
S100A2	S100A8/S100A9 Heterodimer
S100A4	
S100A6	
S100A7	
S100A8	
S100A9	
S100A10	
S100A11	
S100A13	
S100A16	
S100B	
S100P	
TLR-4	
TLR-4/MD-2 Complex	



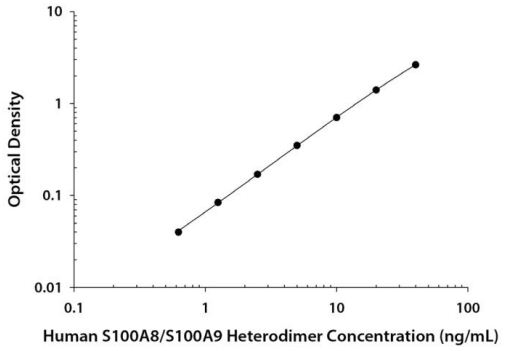


Human serum and conditioned media were analyzed by Western blot and Valukine ELISA. THP-1 human acute monocytic leukemia cells were left unstimulated or treated with PMA for 24 hours prior to harvest, and HepG2 human hepatocellular carcinoma cells were left unstimulated. For the Western blot, serum was diluted 1:100, while conditioned medias were run neat. Samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with sheep anti-human S100A8 (R&D Systems, Catalog # AF4570). The Western blot shows a direct correlation with the ELISA value for these samples.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.004 0.005	0.005	—
0.625	0.044 0.045	0.045	0.040
1.25	0.087 0.091	0.089	0.084
2.5	0.173 0.176	0.175	0.170
5	0.355 0.357	0.356	0.351
10	0.698 0.720	0.709	0.704
20	1.381 1.434	1.408	1.403
40	2.587 2.696	2.642	2.637

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human S100A8/S100A9 Heterodimer Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human S100A8/S100A9 Heterodimer	1 plate
Human S100A8/S100A9 Heterodimer Conjugate	Solution of antibody against human S100A8/S100A9 Heterodimer conjugated to horseradish peroxidase	1 vial
Human S100A8/S100A9 Heterodimer Standard	Recombinant human S100A8/S100A9 Heterodimer in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1-34	A buffered protein base	1 vial
Calibrator Diluent RD5-10	A buffered protein base used to dilute standard and samples	3 vials
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Discard after use. Use a new standard for each assay.</td></tr>
<tr><td>Assay Diluent RD1-34</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD5-10</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital shaker (0.12” orbit) capable of maintaining a speed of 500±50 rpm.
- Test tubes for dilution of standards and samples.

D. SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- RIPA buffer with protease inhibitors

E. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
- Human S100A8/S100A9 Heterodimer is detectable in human saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Tissue Lysates - Tissue samples were lysed prior to assay as described in the Sample Values section. Samples may require dilution with Calibrator Diluent RD5-10.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 × g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Human Milk - Centrifuge for 15 minutes at 1000 × g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Fecal Extract - Fecal samples were prepared prior to assay as described in the Sample Values section. Samples may require dilution with Calibrator Diluent RD5-10.

B. SAMPLE PREPARATION

Human serum samples recommend a 200-fold dilution due to high endogenous levels. A suggested 200-fold dilution can be achieved by adding 20 µL of sample to 180 µL of Calibrator Diluent RD5-10. Complete the 200-fold dilution by adding 10 µL of the

diluted sample to 190 µL Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

Human plasma samples recommend a 100-fold dilution due to high endogenous levels. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

Human saliva samples recommend a 500-fold dilution due to high endogenous levels. A suggested 500-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5-10. Complete the 500-fold dilution by adding 20 µL of the diluted sample to 180 µL Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

Human milk samples recommend at least a 10-fold dilution due to high endogenous levels. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

For tissue lysate and human fecal extract samples, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total tissue lysate protein added is 1-5 µg/well. The suggested range for total fecal extract protein added is 5-50 µg/well. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

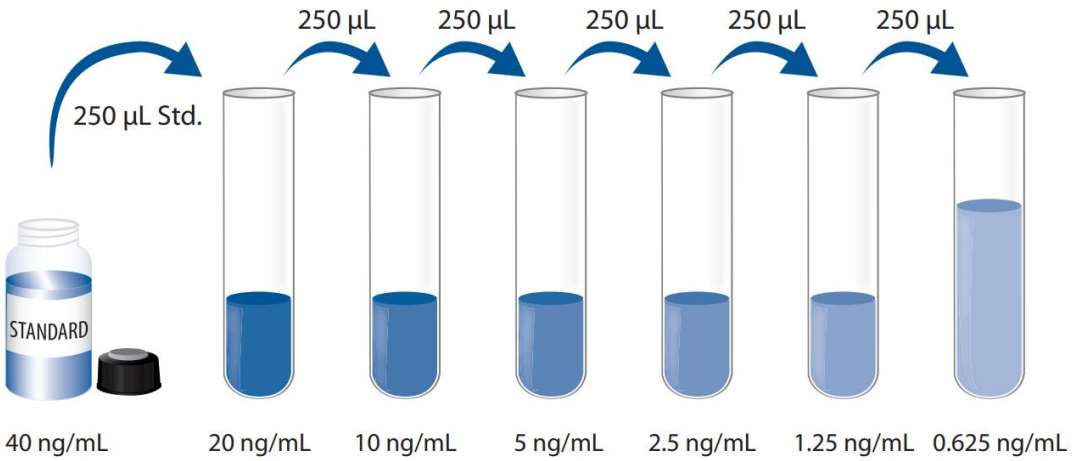
Note: *Human S100A8/S100A9 Heterodimer are found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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

Human S100A8/S100A9 Heterodimer Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human S100A8/S100A9 Heterodimer Standard with Calibrator Diluent RD5-10. This reconstitution produces a stock solution of 40 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

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

Pipette 250 µL of Calibrator Diluent RD5-10 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human S100A8/S100A9 Heterodimer Standard (40 ng/mL) serves as the high standard. Calibrator Diluent RD5-10 serves as the zero standard (0 ng/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

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

Note: *Human S100A8/S100A9 Heterodimer is found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-34 to each well.
4. Add 50 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human S100A8/S100A9 Heterodimer Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
11. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human S100A8/S100A9 Heterodimer concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

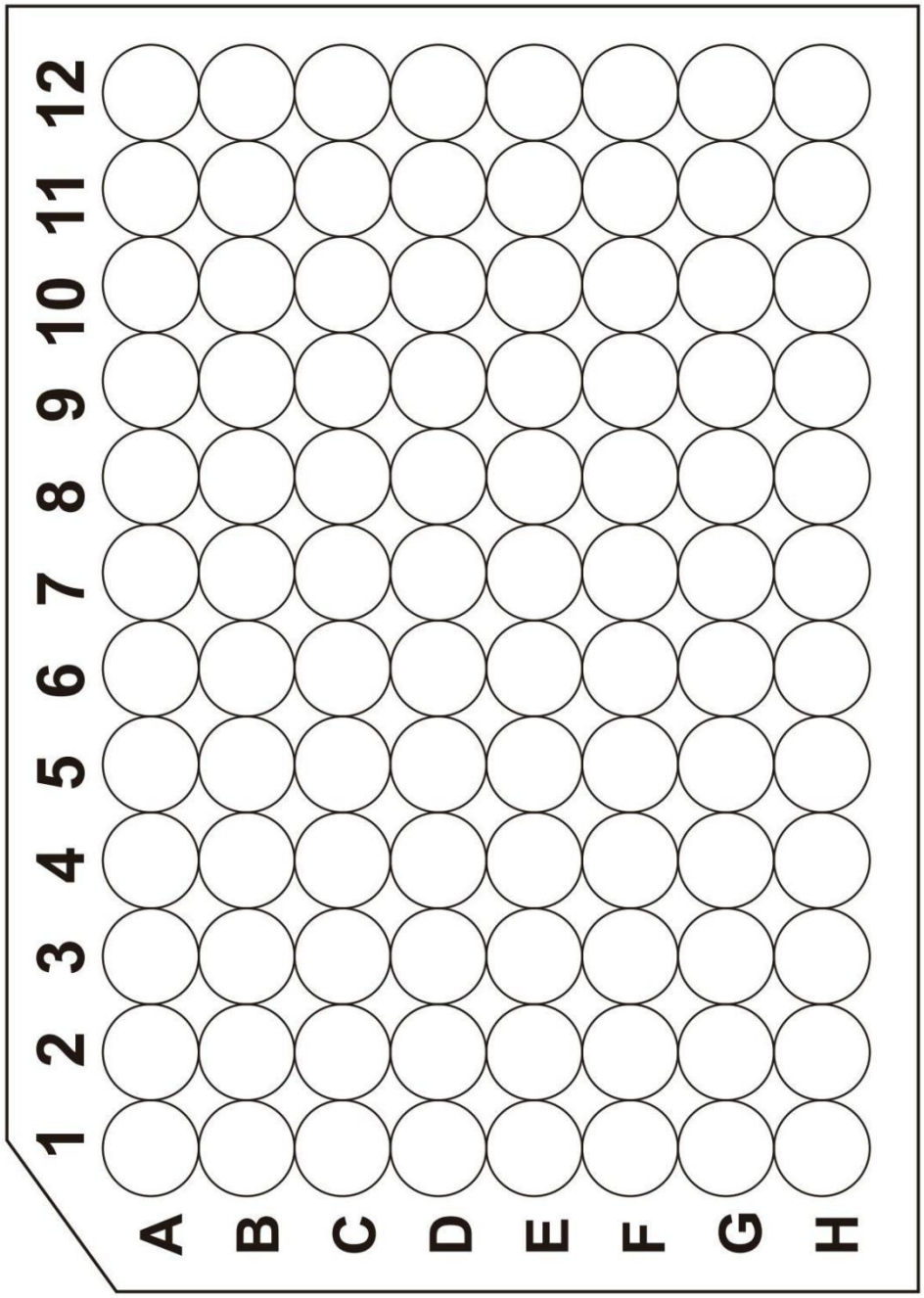
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Averill, M.M. et al. (2012) Arterioscler. Thromb. Vasc. Biol. 32:223.
2. Kerkhoff, C. et al. (2012) Exp. Dermatol. 21:822.
3. Odink, K. et al. (1987) Nature 330:80.
4. Dorin, J.R. et al. (1987) Nature 326:614.
5. Teigelkamp, S. et al. (1991) J. Biol. Chem. 266:13462.
6. Ryckman, C. et al. (2003) J. Immunol. 170:3233.
7. Vogl, T. et al. (2006) Biochim. Biophys. Acta 1763:1298.
8. Vogl, T. et al. (2012) Int. J. Mol. Sci. 13:2893.
9. Siegenthaler, G. et al. (1997) J. Biol. Chem. 272:9371.
10. Volz, H.C. et al. (2012) Basic Res. Cardiol. 107:250.
11. Sunahori, K. et al. (2006) Arthritis Res. Ther. 8:R69.
12. Zhang, L. et al. (2015) Mol. Med. Rep. 11:4093.
13. Narumi, K. et al. (2015) J. Immunol. 194:5539.
14. Chernov, A.V. et al. (2015) J. Biol. Chem. 290:11771.
15. Jin, S. et al. (2014) Exp. Dermatol. 23:938.
16. Vogl, T. et al. (2014) Nat. Commun. 5:4593.
17. Hansson, C. et al. (2014) J. Immunol. Res. 2014:696415.
18. Sekimoto, R. et al. (2012) Biochem. Biophys. Res. Commun. 419:782.
19. Ryu, M-J. et al. (2012) J. Biol. Chem. 287:22948.
20. Schonthaler, H.B. et al. (2013) Immunity 39:1171.
21. Schelbergen, R.F. et al. (2014) Ann. Rheum. Dis. 73 (Suppl 2):116.
22. Damo, S.M. et al. (2013) Proc. Natl. Acad. Sci. USA 110:3841.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 S100A8/S100A9 Heterodimer Valukine™ ELISA 试剂盒

目录号：VAL248

适用于定量检测天然和重组人 S100A8/S100A9 Heterodimer 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景

S100A8（又称 MRP8、钙谷蛋白 A 和 CP-10）和 S100A9（又称 MRP14 和钙谷蛋白 B）是分泌型钙结合蛋白 S100 家族中的促炎成员（1, 2）。10 kDa 的人 S100A8 和 14 kDa 的 S100A9 各含有两个 EF-hand 钙结合基团。人 S100A8 与小鼠和大鼠 S100A8 的氨基酸（amino acid, aa）序列相同度分别为 57%和 61%。人 S100A9 与小鼠和大鼠 S100A9 分别有 57%和 62%的氨基酸序列相同性（3, 4）。在钙或锌存在的情况下，S100A8 和 S100A9 可结合成二硫键连接的同源二聚体和 34-35 kDa 的异源二聚体，即 S100A8/S100A9 异源二聚体或钙蛋白（5-8）。

在炎症部位，S100A8 和 S100A9 在中性粒细胞、单核细胞、许旺细胞和角质细胞中上调（9-15）。类风湿性关节炎滑液以及心血管疾病、特应性皮炎和银屑病关节炎患者血清中的 S100A8/S100A9 异二聚体含量升高（1, 11, 15-17）。其水平与身体脂肪含量和白细胞数量相关（18）。S100A8/S100A9 异二聚体通过受体 RAGE 和 TLR4 发挥作用（13, 19）。异二聚体可促进中性粒细胞渗入炎症部位，并促进单核细胞产生炎性细胞因子（6, 10, 11, 14, 20）。它能促进骨关节炎中骨刺的形成（21）、上调补体 C3（20）、星形胶质细胞的增殖（19），并通过促进 NK 细胞的流入和活化来抑制肿瘤的生长（12，13）。此外，S100A8/S100A9 异二聚体还能与脂肪酸如花生四烯酸结合（9），并能螯合锰，从而限制依赖锰的细菌的生长（22）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人S100A8/S100A9 Heterodimer抗体包被于微孔板上，样品和标准品中的人S100A8/S100A9 Heterodimer会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人S100A8/S100A9 Heterodimer检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断**。
- 该试剂盒适用于细胞培养上清液、组织裂解物、人血清、人血浆、人唾液、人尿液、人乳和人粪便提取物。
- 请在试剂盒有效期内使用。
- 不同试剂盒及不同批号试剂盒的组分不能混用。
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液RD5-10稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（ng/mL）	5.64	12.1	25.3	6.09	12.6	24.6
标准差	0.155	0.370	1.14	0.352	0.606	0.792
CV%	2.7	3.1	4.5	5.8	4.8	3.2

B. 回收率

不同类型样本中掺入检测范围内不同水平的人S100A8/S100A9 Heterodimer，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	85	79-96
裂解液*（n=3）	94	80-104
人尿液*（n=4）	100	91-113
人乳*（n=4）	96	83-103

*测定前稀释样品。

C. 灵敏度

评估 21 次试验，人 S100A8/S100A9 Heterodimer 的最小可检测剂量（MDD）范围为 0.005-0.215 ng/mL，平均 MDD 为 0.086 ng/mL。

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

D. 校正

该免疫测定法以R&D Systems生产的高纯度的*E. coli*表达的重组人S100A8/S100A9 Heterodimer校正。

E. 线性

不同的样本中含有或掺入高浓度的人S100A8/S100A9 Heterodimer，然后用标准品稀释液RD5-10将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基*（n=4）	裂解液（n=3）	人血清*（n=4）	人EDTA血浆*（n=4）	人肝素血浆*（n=4）
1:2	平均值/期待值（%）	104	107	104	106	104
	范围（%）	100-112	99-119	99-109	102-109	101-109
1:4	平均值/期待值（%）	104	110	110	111	110
	范围（%）	99-112	100-120	106-116	102-115	99-119
1:8	平均值/期待值（%）	103	112	111	111	116
	范围（%）	101-105	105-121	107-118	100-117	101-124
1:16	平均值/期待值（%）	99	114	113	104	115
	范围（%）	95-104	103-125	106-116	101-109	101-124

稀释倍数		人唾液*（n=4）	人尿液*（n=4）	人乳*（n=4）	人粪便提取物（n=3）
1:2	平均值/期待值（%）	103	102	103	103
	范围（%）	100-107	101-105	101-107	96-112
1:4	平均值/期待值（%）	105	104	104	103
	范围（%）	102-115	101-109	99-108	94-113
1:8	平均值/期待值（%）	105	105	102	105
	范围（%）	101-115	97-118	99-105	94-111
1:16	平均值/期待值（%）	101	102	100	88
	范围（%）	92-112	87-118	90-105	86-90

*测定前稀释样品。

F. 样本预值

人血清/血浆/唾液/尿液/乳 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人 S100A8/S100A9 Heterodimer。本研究中使用的供体没有病史。

样本类型	平均值（ng/mL）	范围（ng/mL）	标准差（ng/mL）
人血清（n=72）	2015	481-6540	1219
人EDTA血浆（n=40）	473	127-1395	295
人肝素血浆（n=40）	830	298-1640	314
人唾液（n=10）	7271	2137-18,960	5336
人尿液（n=10）	102	0.858-359	99.7
人乳（n=10）	1308	34.0-5720	1876

细胞培养上清：

人外周血白细胞（PBL；1×10^6）在RPMI1640培养基中培养，并加入10%的胎牛血清。细胞在不刺激或用10 µg/mL PHA刺激1或5天后。取细胞培养上清液测定人S100A8/S100A9 Heterodimer的浓度。

条件	1 天（ng/mL）	5 天（ng/mL）
未刺激	399	575
刺激	588	1091

THP-1人急性单核白血病细胞在含有10%胎牛血清和2 mM L-谷氨酰胺的RPMI 1640中培养。细胞在未刺激或用200 nM PMA刺激后培养至汇合。取细胞培养上清液，测定人S100A8/S100A9 Heterodimer的浓度，分别为11.6 ng/mL和38.0 ng/mL。

组织裂解物 - 人结肠组织样品在RIPA缓冲液和蛋白酶抑制剂中制备。工作缓冲液保持在冰上，并在1天内使用。取组织裂解物，测定人S100A8/S100A9 Heterodimer的浓度。

条件	ng/mL
人结肠，正常	14,700
人结肠，癌症	11,215

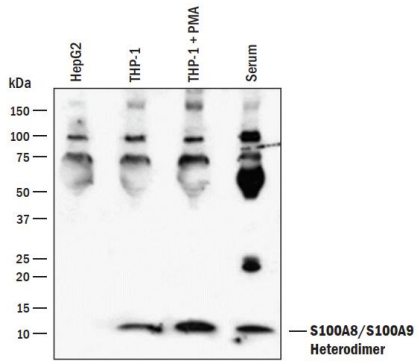
人粪便提取物 - 向100 mg样品中加入5 mL提取液（0.1 M Tris、0.015 M NaCl、1.0 M尿素、1.0 mM $CaCl_2$、0.1 M柠檬酸一水合物、5 mg/mL BSA 和0.25%硫酸庆大霉素，pH 8.0）来提取三种人粪便样品。将样品涡旋混合10分钟，并通过0.2μm滤膜过滤。测定人S100A8/S100A9 Heterodimer浓度，结果为81.2 ng/mL、8.71 ng/mL和1175 ng/mL。

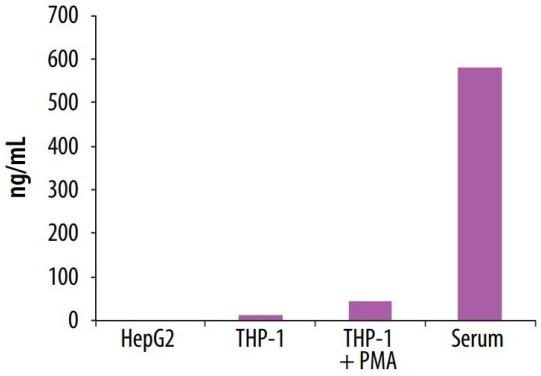
G. 特异性

检测方法识别天然和重组人S100A8/S100A9 Heterodimer。

以下列出的因子在标准品稀释液RD5-10中以500 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人S100A8/S100A9 Heterodimer对照品中以500 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:	Recombinant mouse:
RAGE	S100A8
S100A1	S100A9
S100A2	S100A8/S100A9 Heterodimer
S100A4	
S100A6	
S100A7	
S100A8	
S100A9	
S100A10	
S100A11	
S100A13	
S100A16	
S100B	
S100P	
TLR-4	
TLR-4/MD-2 Complex	



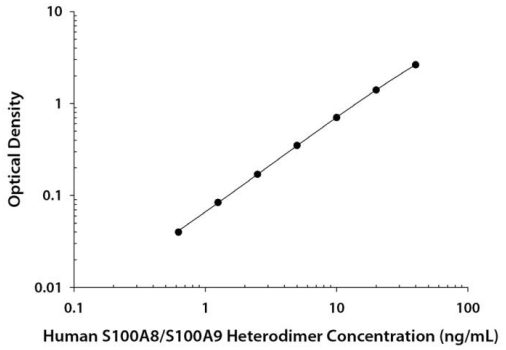


通过Western blot和Valukine ELISA分析人血清和条件培养基。THP-1人急性单核白血病细胞在收获前24小时未受到刺激或用PMA处理，HepG2人肝细胞癌细胞未受刺激。对于蛋白质印迹，血清以1：100稀释，同时将条件培养基直接进行电泳测试。在还原SDS-PAGE条件下分离样品，转移到PVDF膜上，并用绵羊抗人S100A8（R&D Systems，目录 # AF4570）进行免疫印迹。Western印迹显示与这些样品的 ELISA 值直接相关。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(ng/mL)	O.D.	Average	Corrected
0	0.004 0.005	0.005	—
0.625	0.044 0.045	0.045	0.040
1.25	0.087 0.091	0.089	0.084
2.5	0.173 0.176	0.175	0.170
5	0.355 0.357	0.356	0.351
10	0.698 0.720	0.709	0.704
20	1.381 1.434	1.408	1.403
40	2.587 2.696	2.642	2.637

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human S100A8/S100A9 Heterodimer Microplate	包被抗人S100A8/S100A9 Heterodimer抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human S100A8/S100A9 Heterodimer Conjugate	酶标检测抗人S100A8/S100A9 Heterodimer抗体	1瓶
Human S100A8/S100A9 Heterodimer Standard	重组人S100A8/S100A9 Heterodimer标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1-34	检测液	1瓶
Calibrator Diluent RD5-10	标准品稀释液，用于稀释标准品和样品	3瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>使用后丢弃。每次测试使用新的标准品。</td></tr>
<tr><td>检测液RD1-34</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>标准品稀释液RD5-10</td><td>2-8°C 储存，最多 30 天*</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8 °C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

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

D. 组织裂解物样品所需的耗材

- 含蛋白酶抑制剂的RIPA缓冲液

E. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。
- 人唾液中可检测到S100A8/S100A9 Heterodime。在进行此测定时，请采取预防措施，防止试剂盒试剂污染。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5-10稀释。

组织裂解物 - 组织样品在检测前按“样品预值”部分所述进行裂解。样品可能需要使用标准品稀释液RD5-10稀释。

血清 - 使用血清分离管（SST），让样本在室温下凝固30分钟，然后在1000 × g 的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融。样本可能需要用标准品稀释液RD5-10稀释。

血浆 - 使用肝素或EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-10稀释。

注： *柠檬酸盐血浆在本检测中未经验证。*

唾液 - 将唾液收集到管中，以10,000 × g离心5分钟。收集上清液，立即检测或分装并储存在≤-20°C。避免反复冻融。样品可能需要用标准品稀释液RD5-10稀释。

尿液 - 采集晨尿（中段），直接排入无菌容器。离心去除颗粒物，立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-10稀释。

人乳 - 在2-8 °C下，以1000 × g离心15分钟。收集上清液，重复此过程总共3次。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-10稀释。

粪便提取物 - 粪便样品在测定之前按照“样品预值”部分所述制备。样品可能需要用标准品稀释液RD5-10稀释。

B. 样品准备

人血清样品由于内源性较高，建议进行 200 倍稀释。建议的 200 倍稀释方式：将 20 µL 样品加入到 180 µL 标准品稀释液 RD5-10，再将 10 µL 稀释后的样品加入 190 µL 标准品稀释液 RD5-10 来完成 200 倍稀释。最佳稀释倍数应由用户自行确定。

人血浆样品由于内源性水平高，建议进行 100 倍稀释。建议的 100 倍稀释方式：将 10 µL 样品加入到 990 µL 标准品稀释液 RD5-10 中。最佳稀释倍数应由用户自行确定。

人唾液样品由于内源性较高，建议进行 500 倍稀释。建议的 500 倍稀释方式：将 10 µL 样本加入 490 µL 标准品稀释液 RD5-10 来实现。再将 20 µL 稀释后的样本加入到 180 µL 标准品稀释液 RD5-10 中，完成 500 倍稀释。最佳稀释倍数应由用户自行确定。

人乳样品于内源性较高，建议进行 10 倍稀释。建议的 10 倍稀释方式：将 20 µL 样品加入到 180 µL 标准品稀释液 RD5-10 中。最佳稀释倍数应由用户自行确定。

对于组织裂解物和人粪便提取物样品，建议使用总蛋白测定法定量检测样品蛋白质浓度。推荐的总组织裂解物蛋白添加量范围为 1-5 µg/孔。推荐的总粪便提取物蛋白添加范围为 5-50 µg/孔。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

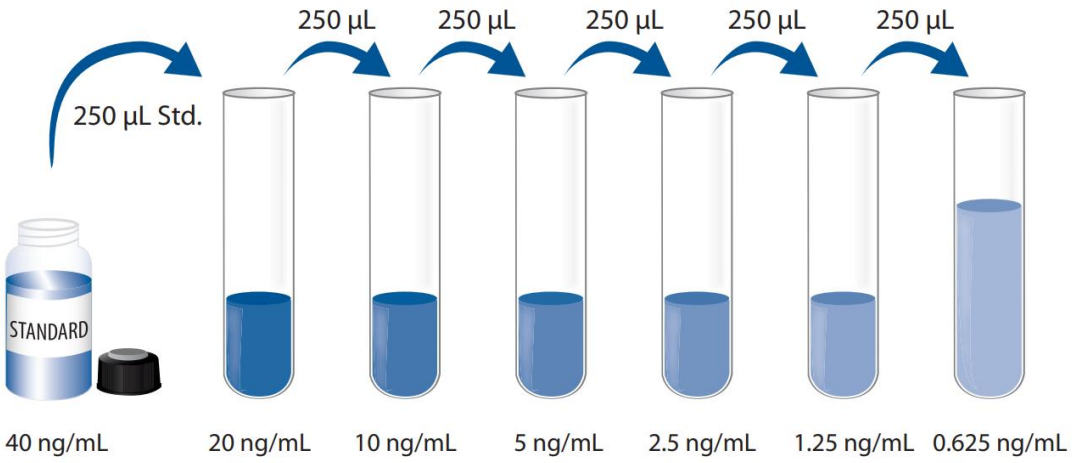
注：*唾液中含有S100A8/S100A9 Heterodimer。建议使用口罩和手套以防止试剂盒试剂受污染。*

洗涤液（1×）：浓缩洗涤液可能含有结晶，将其放置室温，轻摇混匀，待结晶完全溶解。将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人S100A8/S100A9 Heterodimer标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD5-10复溶人S100A8/S100A9 Heterodimer标准品，得到浓度为40 ng/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将250 µL标准品稀释液RD5-10移入每个管中。使用标准品储备母液稀释（如下）。在每次转移之前，将每个管彻底混合。未稀释的标准品作为标准曲线的最高标点（40 ng/mL）。标准品稀释液RD5-10作为标准曲线的零点（0 ng/mL）。



D. 技术小提示

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

VII.操作步骤

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

注：*人唾液中存在人S100A8/S100A9 Heterodimer。建议佩戴口罩和手套以防止试剂盒试剂受污染。*

1. 按照上一节的说明，准备好所有需要的试剂和标准品。
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口。
3. 向每个孔中加入50 µL的检测液RD1-34。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置。
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体。
6. 在每个微孔内加入200 µL人S100A8/S100A9 Heterodimer酶标检测抗体。用新的封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，室温孵育2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确。
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人S100A8/S100A9 Heterodimer浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Averill, M.M. et al. (2012) Arterioscler. Thromb. Vasc. Biol. 32:223.
2. Kerkhoff, C. et al. (2012) Exp. Dermatol. 21:822.
3. Odink, K. et al. (1987) Nature 330:80.
4. Dorin, J.R. et al. (1987) Nature 326:614.
5. Teigelkamp, S. et al. (1991) J. Biol. Chem. 266:13462.
6. Ryckman, C. et al. (2003) J. Immunol. 170:3233.
7. Vogl, T. et al. (2006) Biochim. Biophys. Acta 1763:1298.
8. Vogl, T. et al. (2012) Int. J. Mol. Sci. 13:2893.
9. Siegenthaler, G. et al. (1997) J. Biol. Chem. 272:9371.
10. Volz, H.C. et al. (2012) Basic Res. Cardiol. 107:250.
11. Sunahori, K. et al. (2006) Arthritis Res. Ther. 8:R69.
12. Zhang, L. et al. (2015) Mol. Med. Rep. 11:4093.
13. Narumi, K. et al. (2015) J. Immunol. 194:5539.
14. Chernov, A.V. et al. (2015) J. Biol. Chem. 290:11771.
15. Jin, S. et al. (2014) Exp. Dermatol. 23:938.
16. Vogl, T. et al. (2014) Nat. Commun. 5:4593.
17. Hansson, C. et al. (2014) J. Immunol. Res. 2014:696415.
18. Sekimoto, R. et al. (2012) Biochem. Biophys. Res. Commun. 419:782.
19. Ryu, M-J. et al. (2012) J. Biol. Chem. 287:22948.
20. Schonthaler, H.B. et al. (2013) Immunity 39:1171.
21. Schelbergen, R.F. et al. (2014) Ann. Rheum. Dis. 73 (Suppl 2):116.
22. Damo, S.M. et al. (2013) Proc. Natl. Acad. Sci. USA 110:3841.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置