Speedy™ Mouse RAGE One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE60031

Size: 96T

Sensitivity: 1.4 pg/mL

Range: 31.25-2000 pg/mL, 62.5-4000 pg/mL

Usage: For the quantitative detection of mouse RAGE concentrations in serum, plasma, cell culture supernatant and tissue lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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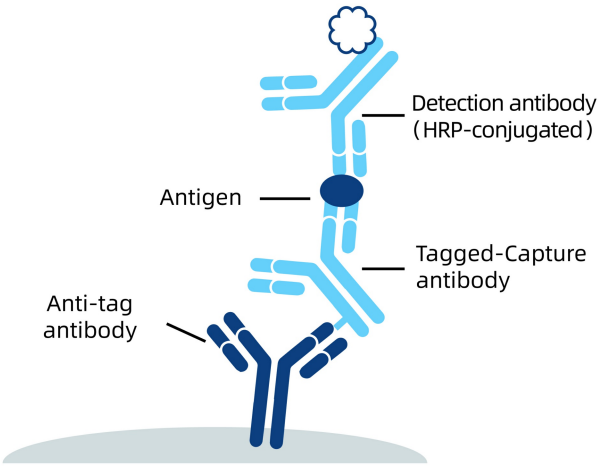
1. Background

RAGE (receptor for advanced glycation endproducts), also called AGER, is a transmembrane receptor of the immunoglobulin super family. It mediates interactions of advanced glycosylation end products (AGE) which accumulate in vascular tissue in aging in diabetes. Acts as a mediator of vascular inflammation such as atherosclerosis and a complication of diabetes. AGE/RAGE signal regulates production or expression of TNF-alpha, oxidative stress, and endothelial dysfunction in type 2 diabetes. Interaction with S100A12 on endothelium, mononuclear phagocytes, and lymphocytes triggers cellular activation. Interaction with S100B after myocardial infarction regulates myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling. ABPP-initiated RAGE signaling, especially stimulation of P38 mitogen-activated protein kinase (MAPK), delivers ABPP as a complex with RAGE to the intraneuronal. RAGE has higher expression in lung tissues, in particular in alveolar type I cells, and is lost in idiopathic pulmonary fibrosis (IPF).

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Additional Diluent AT-60031 - 6 mL/bottle. Only for serum and plasma samples.	1 bottle	
Sample Diluent PT 3-ef - 30 mL/bottle. For mouse serum and plasma samples.	1 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle. For cell culture supernatant and tissue lysate.	2 bottles	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 15 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for mouse serum and plasma; 1:400 or 1:800 is recommended for cell culture supernatant; 1:4 to 1:64,000 is recommended for tissue lysate.

7.4 Standard Serial Dilution:

For mouse serum and plasma, add 1 mL Sample Diluent PT 3-ef in protein standard.

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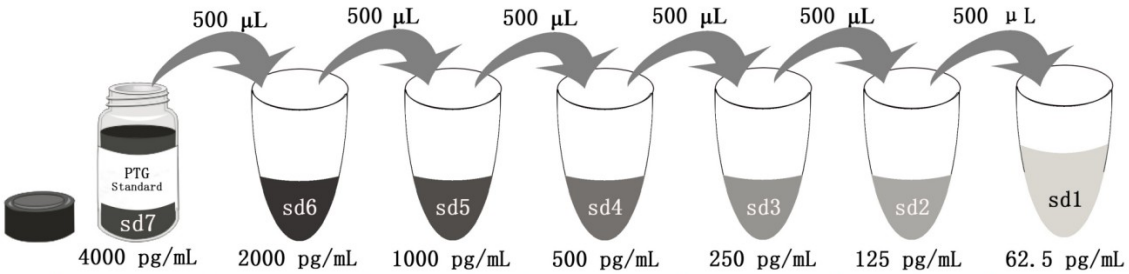
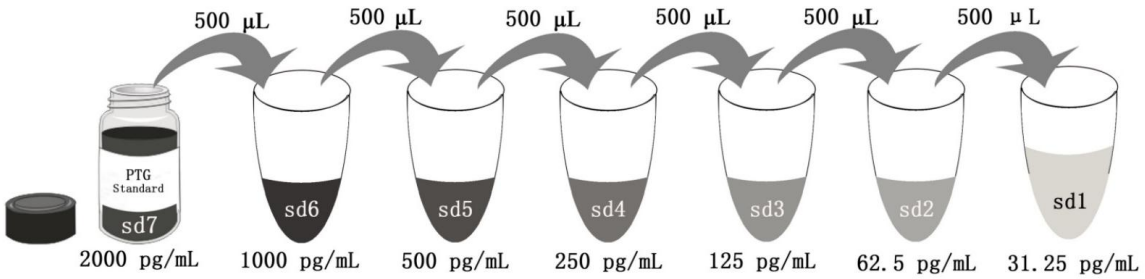


Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-ef	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

For cell culture supernatant and tissue lysate, add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). 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.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group:

For serum or plasma, add 25 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant and tissue lysate, no need to add Additional Diluent, directly follow the next step.

8.3 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.4 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.6 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.7 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.8 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.9 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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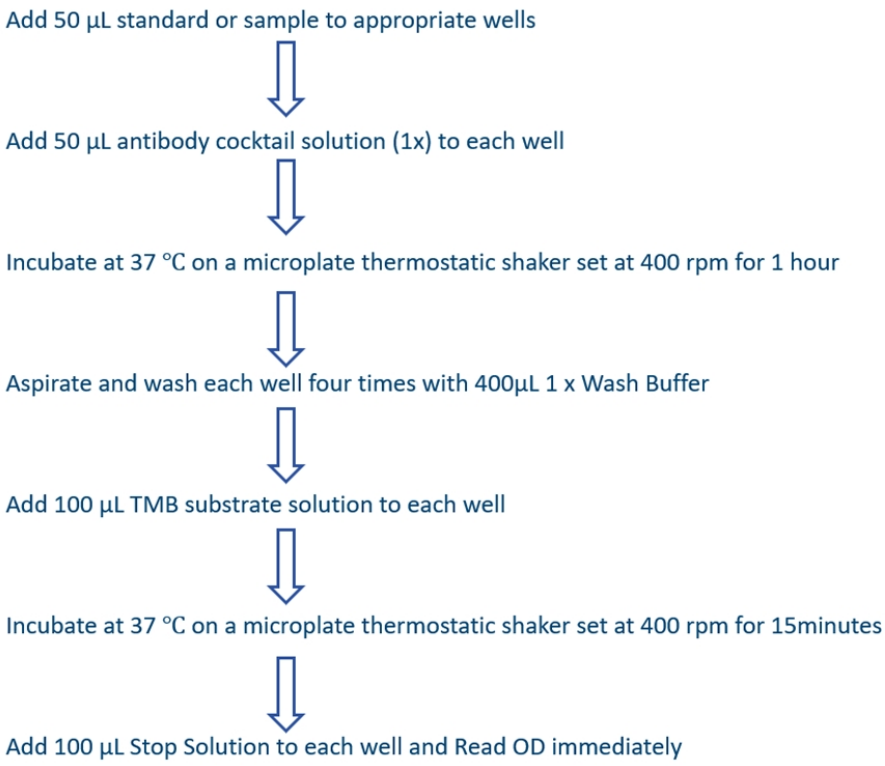

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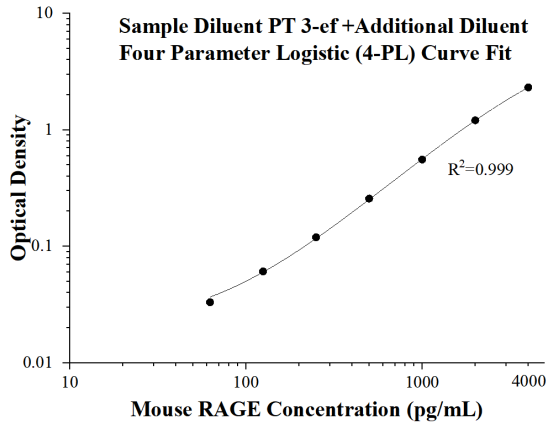
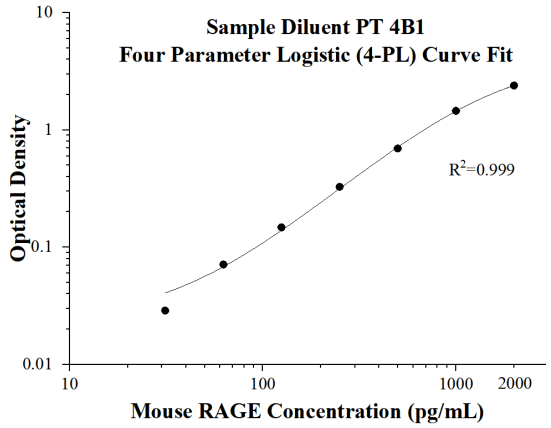
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0091 0.0082	0.00865	-
62.5	0.0418 0.0417	0.04175	0.0331
125	0.0698 0.0694	0.0696	0.06095
250	0.131 0.1256	0.1283	0.11965
500	0.2683 0.2636	0.26595	0.2573
1000	0.5574 0.5732	0.5653	0.55665
2000	1.2061 1.2303	1.2182	1.20955
4000	2.3061 2.3438	2.32495	2.3163



(pg/mL)	O.D	Average	Corrected
0	0.0281 0.0281	0.0281	-
31.25	0.0572 0.0566	0.0569	0.0288
62.5	0.1007 0.098	0.09935	0.07125
125	0.1797 0.1716	0.17565	0.14755
250	0.3516 0.3596	0.3556	0.3275
500	0.7132 0.7324	0.7228	0.6947
1000	1.4849 1.4817	1.4833	1.4552
2000	2.4048 2.4462	2.4255	2.3974

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,026.4	12.8	1.2
2	8	252.9	1.5	0.6
3	8	130.1	2.0	1.5

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,034.6	16.0	1.5
2	16	254.2	4.2	1.7
3	16	132.3	3.0	2.3

9.3 Recovery

The recovery of mouse RAGE spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:4	112	106-116
Cell culture supernatant	1:1,600	106	98-117
Tissue lysate	1:16	88	78-103

9.4 Sample values

Mouse serum - mouse serum samples were evaluated for the presence of mouse RAGE in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	994.5	391.0-2,148.0

Cell culture supernatant - Mouse lung from individual mice were removed and rinsed in PBS and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for 6 days. An aliquot of the cell culture supernate was removed, assayed for mouse RAGE, and measured 667.3 **ng/mL**.

Tissue lysate

	Mouse RAGE (**ng/mL**)	Total protein (**mg/mL**)
Mouse spleen tissue lysate	4.9	6.4
Mouse lung tissue lysate	30,779.1	5.9

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9.5 Sensitivity

The minimum detectable dose of mouse RAGE is 1.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The cell culture supernatant was initially diluted 1:200. The tissue lysate was initially diluted 1:16,000.)

		Mouse serum (Sample Diluent PT 3-ef)	Cell culture supernatant (Sample Diluent PT 4B1)	Tissue lysate (Sample Diluent PT 4B1)
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	101	90	99
	Range (%)	94-109	80-101	96-103
1:8	Average% of Expected	87	90	101
	Range (%)	82-92	79-100	95-107
1:16	Average% of Expected	-	88	101
	Range (%)	-	76-99	92-110

9.7 Specificity

This assay recognizes natural and recombinant mouse RAGE.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

HMGB1

S100B

Recombinant mouse:

CD36/SR-B3

Galectin-3

Olr1

S100A10

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10. References

1. Neeper, M et al. The Journal of biological chemistry vol. 267,21 (1992): 14998-5004.

2. Hofmann, M A et al. Cell vol. 97,7 (1999): 889-901.

3. Queisser, Markus A et al. American journal of respiratory cell and molecular biology vol. 39,3 (2008): 337-45.

4. Gao, Xue et al. American journal of physiology. Heart and circulatory physiology vol. 295,2 (2008): H491-8.

5. Takuma, Kazuhiro et al. Proceedings of the National Academy of Sciences of the United States of America vol. 106,47 (2009): 20021-6.

6. Fang, Fang et al. FASEB journal : official publication of the Federation of American Societies for Experimental Biology vol. 24,4 (2010): 1043-55.

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