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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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RAGE ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of RAGE

Catalogue number LDG00004E

For Research Use or Further Manufacturing Only.



96 wells





Store at 2-8℃

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Leadgene® RAGE ELISA Kit

1. Introduction

The receptor for advanced glycation end products (RAGE) is a multiligand cell surface receptor belonging to the immunoglobulin superfamily. RAGE is involved in various cellular processes, including inflammatory responses, oxidative stress regulation, and signal transduction, and has been associated with multiple pathological conditions such as cancer, cardiovascular diseases, and chronic inflammatory disorders. Reliable detection and quantification of RAGE are important for basic research and disease-related studies. Leadgene® RAGE ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of RAGE levels in sample solution. The RAGE ELISA Kit is for research use only (RUO). Not suitable for use in diagnostic or therapeutic procedures.

2. Test principle

RAGE ELISA Kit is used to detect RAGE in samples by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-RAGE monoclonal antibody to the solid phase. RAGE in the samples conjugates on solid phase and then react with the HRP conjugated mouse anti-RAGE monoclonal antibody. Subsequent wash steps will residually unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of RAGE level is accomplished by comparing the absorbance with standard curve.

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3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
Antibody-coated microplate Stripwell microplate with 96 anti-RAGE monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard Lyophilized	1 vial (Lyophilized form)	**Refer to the vial label for reconstitution volume.** Reconstitute by adding Standard reconstitution buffer to be a stock solution of 50 ng/mL. (see procedure, section 8.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	1 vial (1.1 mL)	**Ready** for use
Sample and Antibody diluent buffer Buffered solution with preservatives	1 vial (20 mL)	**Ready** for use
Assay buffer Buffered solution with preservatives	1 vial (6 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti-RAGE monoclonal antibody in buffered protein solution with preservatives	1 vial (115 μL)	**Dilute** 100 x with Sample and Antibody diluent buffer (see reagent preparation, section 5.A)
10 X wash buffer 10-fold concentrated solution of buffered surfactant with preservatives	1 vial (20 mL)	**Dilute** 10 x with distilled water (see reagent preparation, section 5.B)
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	**Ready** for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	**Ready** for use
Microplate sealing film	2 sheets	N/A

4. Materials required but not provided

(1) High quality distilled water
(2) 10 mL graduated pipettes
(3) 5 μL to 1000 μL adjustable single-channel micropipettes with disposable tips
(4) 50 μL to 300 μL adjustable multi-channel micropipettes with disposable tips
(5) Multi-channel micropipette reservoir
(6) Disposable microcentrifuge tubes

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(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) Timer
(9) Magnetic stirrer
(10) Vortex mixer
(11) Washer for microplates
(12) Stripwell microplate spectrophotometer capable of reading at 450 nm
(13) Clean paper towels
(14) Disposable gloves
(15) Discard container for bio-medical waste

5. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

(1) **Working HRP-antibody conjugate (1 X):** Dilute 1 volume of **HRP-antibody conjugate** with 99 volumes of **Sample and Antibody diluent buffer** and homogenize by vortex.

(2) **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Storage and expiration date of reagents

- Before opened, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8℃ after used, except the reconstituted standard, it must be stored at -80℃.
- Avoid multiple freeze-thaw cycles of the frozen standard, and if stored properly at -80℃, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8℃ in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

7. Precautions & warnings

To obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those people who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains H_2SO_4) contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.

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- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Bring all the reagents and specimens to 15-30℃ prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Procedure

(1) Evaluate the number of stripwell required to test the samples. Put the stripwells at room temperature (15-30℃) before use. The unused strips should be resealed in the bag and stored at 2-8℃. Each standard, blank, and sample should be assayed in duplicate.

(2) Standard and sample preparation:

• **Standard preparation (in microcentrifuge tubes):**

- **Refer to the vial label for the reconstitution volume.** Reconstitute the lyophilized standard with Standard Reconstitution Buffer to a final concentration of 50 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C.
- Add 450 µL Sample and Antibody diluent buffer to 50 µL of 50 ng/mL standard to make a 5000 pg/mL standard (Tube 1).
- Adding 200 µL of Sample and Antibody diluent buffer to 200 µL of 5000 pg/mL standard to make a 2500 pg/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only contains Sample and Antibody diluent buffer.

• **Sample preparation:**

- 50 µL Sample. If the initial assay found samples contain RAGE higher than the highest standard, the samples can be diluted with Sample and Antibody diluent buffer and then re-assay the samples.
- Add 50 µL of standards, blanks or samples into RAGE ELISA stripwell microplates (see Table 1), then add 50 µL Assay buffer immediately. Cover with microplate sealing film and incubate sealed plate at RT for 2 hours.
- Remove the sealing film, aspirate the liquid from each well and then wash the plate two times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate sealed plate at RT for 1 hour in the dark.

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- Remove the sealing film, aspirate the liquid from each well and then wash the plate four times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.
- Add 100 µL of TMB into each well. Incubate for 10 minutes at room temperature (15-30℃) in the dark.
- Add 50 µL Stop solution into each well.
- Read the absorbencies immediately at 450 nm after the Stop solution is added.

Table 1 An example of orientation of standards, blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (5000 pg/mL)	Standard 1 (5000 pg/mL)	Sample 1	Sample 5
B	Standard 2 (2500 pg/mL)	Standard 2 (2500 pg/mL)	Sample 1	Sample 5
C	Standard 3 (1250 pg/mL)	Standard 3 (1250 pg/mL)	Sample 2	Sample 6
D	Standard 4 (625 pg/mL)	Standard 4 (625 pg/mL)	Sample 2	Sample 6
E	Standard 5 (312.5 pg/mL)	Standard 5 (312.5 pg/mL)	Sample 3	Sample 7
F	Standard 6 (156.25 pg/mL)	Standard 6 (156.25 pg/mL)	Sample 3	Sample 7
G	Standard 7 (78.125 pg/mL)	Standard 7 (78.125 pg/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

9. Internal quality control

- The average absorbance of Blank: ≦ 0.15
- The average absorbance of highest concentration of standard (5000 pg/mL): ≧ 1.5

10. Calculation of results

- The standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis) using four-parameter logistic (4-PL) curve fit.
- The RAGE concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 5000 pg/mL, the samples should be diluted with Sample and Antibody diluent buffer and re-assay again.

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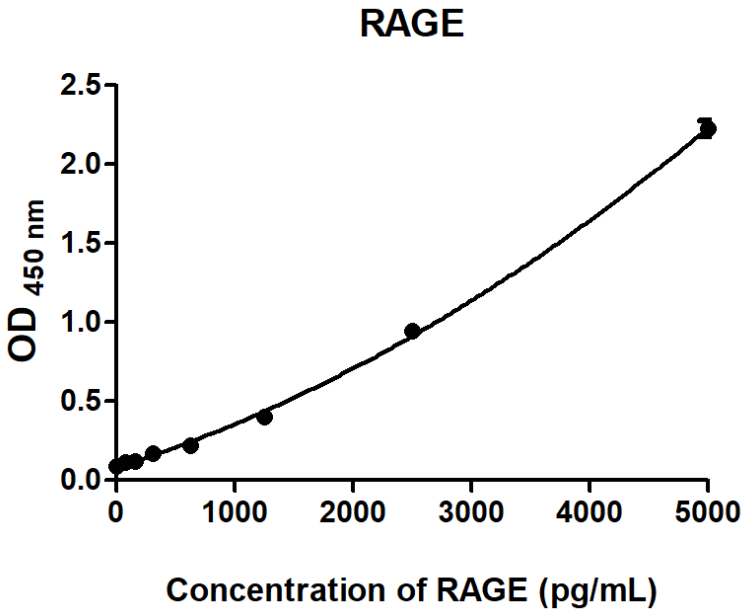
Typical data

The following data are for demonstration only

Standard	RAGE (pg/mL)	OD 450 nm	
1	5000	2.273	2.175
2	2500	0.976	0.915
3	1250	0.412	0.39
4	625	0.231	0.208
5	312.5	0.176	0.164
6	156.25	0.12	0.115
7	78.125	0.11	0.111
Blank	0	0.096	0.087



11. Assay limitations

- Sample should be centrifuged to remove debris.

12. Performance characteristics

Sensitivity

- The limit of detection (LoD) of this RAGE ELISA Kit is 78 pg/mL.
- The limit of quantification (LoQ) of this RAGE ELISA Kit is 344 pg/mL.

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