Datasheet for ABIN625423

RAGE ELISA Kit

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Overview

Quantity:	96 tests
Target:	RAGE (AGER)
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	15-10000 pg/mL
Minimum Detection Limit:	15 pg/mL
Application:	ELISA

Product Details

Purpose:	Mouse RAGE ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30L, CD30, CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CSF, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-
Sensitivity:	15 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data

Product Details

Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	RAGE (AGER)
Alternative Name:	RAGE / AGER (AGER Products)
Background:	MAPK/MAK/MRK overlapping kinase (EC 2.7.11.22) (MOK protein kinase) (Serine/threonine kinase 30)
Gene ID:	26448
UniProt:	Q9WVS4
Pathways:	Carbohydrate Homeostasis, Toll-Like Receptors Cascades, Smooth Muscle Cell Migration, S100 Proteins

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C.

4. Add 100 µL of prepared biotin antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µL of prepared Streptavidin solution to each well.
7. Incubate 45 min at RT.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25°C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent (Item E) is used for dilution of serum/plasma/culture supernatants. 3. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µl 1x Assay Diluent (Item E) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 100 µl RAGE standard solution from the vial of Item C, into a tube with 400 µl 1x Assay Diluent to prepare a 10,000 pg/ml standard solution. Pipette 400 µl 1x Assay Diluent into each tube. Use the 10,000 pg/ml standard solution to produce a Dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. 1x Assay Diluent serves as the zero standard (0 pg/ml). 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 8,000-fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent to prepare a 100-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix through and then pipette 100 µl of prepared 100-fold diluted solution into a tube with 8 ml 1x Assay Diluent to prepare a final 8,000 fold diluted HRP-Streptavidin solution.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash

Application Details

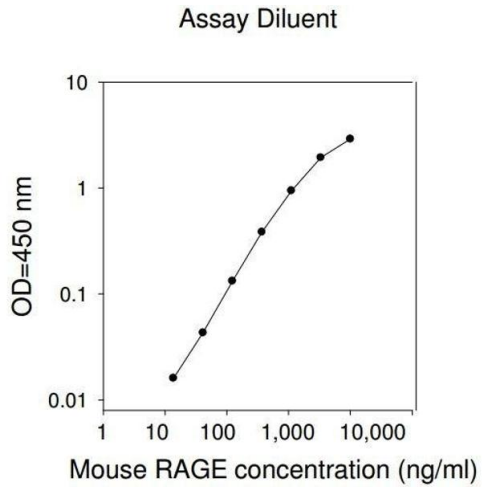
	Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Choi, Suh, Kim, Hong, Park, Chon: "Glabridin Alleviates the Toxic Effects of Methylglyoxal on Osteoblastic MC3T3-E1 Cells by Increasing Expression of the Glyoxalase System and Nrf2/HO-1 Signaling and Protecting Mitochondrial Function." in: **Journal of agricultural and food chemistry**, Vol. 64, Issue 1, pp. 226-35, (2016) (PubMed).



ELISA

Image 1.