canine RAGE

Catalog Number: DY4750

This DuoSet ELISA Development kit contains the basic components required for the development of sandwich ELISAs to measure natural and recombinant canine Receptor for Advanced Glycation End Products (RAGE). DuoSets are designed for the analysis of cell culture supernates. Other sample types, such as serum and plasma, need to be validated prior to use in this DuoSet.[1] Each kit contains sufficient materials to run ELISAs on approximately fifteen 96-well plates, provided that the following conditions are met:[2]

- The assay is run as summarized in the General ELISA protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product.

フナコシ株式会社
試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775
e - mail : reagent@funakoshi.co.jp

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MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody (Part 843012, 1 vial) - 144 μg/mL of sheep anti-canine RAGE when reconstituted with 1.0 mL of PBS. After reconstitution, store at 2 - 8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.[3] Dilute to a working concentration of 0.8 μg/mL in PBS,[4] without carrier protein.

Detection Antibody (Part 843013, 1 vial) - 72 μg/mL of biotinylated sheep anti-canine RAGE when reconstituted with 1.0 mL of Reagent Diluent (see Solutions Required section). After reconstitution, store at 2 - 8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.[3] Dilute to a working concentration of 400 ng/mL in Reagent Diluent.[4]

Standard (Part 843014, 3 vials) - Each vial contains 85 ng/mL of recombinant canine RAGE when reconstituted with 0.5 mL of Reagent Diluent (see Solutions Required section). Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Store reconstituted standard at 2 - 8° C or aliquot and store at -70° C for up to 2 months.[3] A seven point standard curve using 2-fold serial dilutions in Reagent Diluent, and a high standard of 2000 pg/mL is recommended.

Streptavidin-HRP (Part 890803, 1 vial) - 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Store at 2 - 8° C for up to 6 months after initial use.[3] **DO NOT FREEZE**. Dilute to the working concentration specified on the vial label using Reagent Diluent (see Solutions Required section).[4]

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 - 7.4, 0.2 μm filtered.

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2 - 7.4 (R&D Systems Catalog # WA126).

Reagent Diluent[1] - 1% BSA[5] in PBS, pH 7.2 - 7.4, 0.2 μm filtered (R&D Systems Catalog # DY995). **Quality of BSA is critical (see Technical Hints).**

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems Catalog # DY999).

Stop Solution - 2 N H_2SO_4 (R&D Systems Catalog # DY994).

Tween is a registered trademark of ICI Americas.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate[6] with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three 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 for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 μL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 μL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 μL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

TECHNICAL HINTS AND LIMITATIONS

- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- We recommend the use of R&D Systems' Reagent Diluent (Catalog # DY995) or the use of Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045), to prepare your own Reagent Diluent.
- This DuoSet should not be used beyond the expiration date on the label.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2 - 8° C or be prepared fresh daily.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

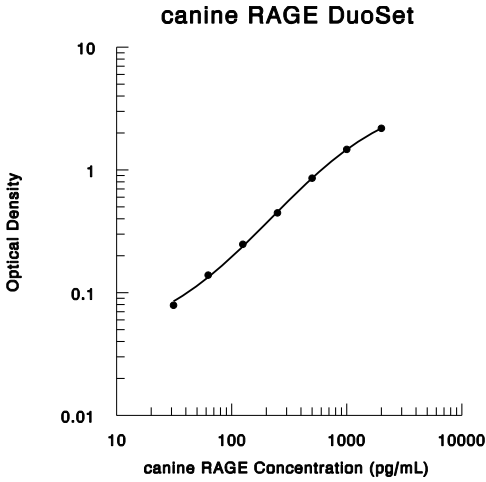
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the RAGE 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.

TYPICAL DATA

This standard curve is only for demonstration purposes.

A standard curve should be generated for each set of samples assayed.

The graph below represents typical data generated when using this canine RAGE DuoSet. The standard curve was calculated using a computer generated 4-PL curve-fit.



SPECIFICITY

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

Recombinant mouse:	**Recombinant rat:**
RAGE/Fc Chimera	RAGE/Fc Chimera

A sample containing 12.5 ng/mL of recombinant human RAGE/Fc Chimera reads as 65 pg/mL (0.5% cross-reactivity).

CALIBRATION

This DuoSet is calibrated against a highly purified NS0-expressed recombinant canine RAGE produced at R&D Systems.

[1]If assaying sample types other than cell culture supernates, each laboratory should develop and validate its own diluent. We suggest starting with PBS supplemented with 10 - 50% fetal calf serum. The diluent must not be used to dilute the Detection Antibody or the Streptavidin-HRP.

[2]Individual results may vary due to differences in technique, plasticware and water sources.

[3]Provided this is within the expiration date of the kit.

[4]Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

[5]All buffers containing BSA must be stored at 2 - 8° C.

[6]Costar EIA Plate (Costar Catalog # 2592 or R&D Systems Catalog # DY990) is suggested. R&D Systems ELISA Plate Sealers (Catalog # DY992) are also available.

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USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420

China | R&D Systems China Co., Ltd.
25/G Hua Min Empire Plaza, 726 West Yan An Road
Shanghai PRC 200050
TEL: (800) 988-1270 FAX: 86 (21) 52371001