Human RAGE-expressing HEK293 Cell Line

CircuLex RAGE/HEK293 Cell Line

Cat# CY-C8250

Intended Use

The CycLex Research Product CircuLex RAGE/HEK293 Cell Line was designed for studying RAGE signaling pathway. The cell line was derived from HEK293 cell line, stably expressing human RAGE, which has 6X his tagged at C-terminus.

Applications for this cell line:
1) Detecting antibodies or proteins binding to RAGE.
2) Screening inhibitors of RAGE-ligand interaction on cell surface.

This cell line is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

• Upon receipt store at liquid N_2. See Shipping and Storage section, page 2.

Introduction

Receptor for Advanced Glycation End product (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules that is expressed in a variety of cell lines, including endothelial cells, smooth muscle cells, mononuclear phagocytes, pericytes, neurons, cardiac myocytes, mesangial cells and hepatocytes (1, 2).

RAGE interacts with different structures to transmit a signal into the cell and recognizes three-dimensional structures rather than specific amino acid sequences. Therefore, RAGE seems to fulfill the requirements of a pattern-recognition receptor. As a member of the immunoglobulin superfamily, it interacts with a diverse class of ligands, including Advanced Glycation End products (AGEs) (3, 4), HMGB1 (also known as Amphoterin) (5), amyloid β-peptide (6), amyloid A (7), leukocyte adhesion receptors (8), prions (9), Escherichia coli curli operons (10), β-sheet fibrils (11) and several members of the S100 protein superfamily including S100/calgranulins (12). Thus RAGE may have potential involvement in several pathological processes including inflammation, diabetes, Alzheimer's disease (AD), systemic amyloidosis, and tumor growth (13). RAGE may also mediate physiological functions, such as neuronal outgrowth, survival, and regeneration, and play a part in pro-inflammatory reactions (12-14, 15).

The activation of RAGE initiates nuclear factor kappa B (NF-κB) (16,17) and mitogen-activated protein kinase (MAPK) pathways (18). Additionally, RAGE-mediated cellular stimulation promotes increased expression of the receptor itself. This positive feedback loop, characterized by ligand-receptor interaction followed by increased expression of the receptor, suggests that RAGE functions as a propagation and perpetuation factor: the two-hit model of RAGE engagement is based on this finding (19).

Components

The RAGE/HEK293 Cell Line is supplied in one vial containing approximately 2×10^6 cells in 1 ml of freezing medium (complete growth medium w/ 10% Dimethyl sulfoxide, DMSO).

1 ml of RAGE/HEK293 Cell Line (~2×10^6 cells)

Shipping and Storage

The RAGE/HEK293 Cell Line is shipped frozen on dry ice. It is strongly recommended that the RAGE/HEK293 Cell Line shall be thawed and propagated as soon as possible following receipt (see "Thawing the RAGE/HEK293 Cell Line" protocol below). If long-term storage of the frozen cells is required, place vial in the vapor phase of liquid nitrogen. Storage of cells directly in liquid nitrogen requires use of protective tubing, such as Nunc Cryoflex™ Tubing. Storage of cells at -80°C is suitable only for short periods of time (a few months), and may result in loss of viability and is not recommended.

Safety Guidelines

This product contains Dimethyl sulfoxide (DMSO), a hazardous material. It is also important to always follow standard tissue culture practices, which include:

- Wearing gloves, safety glasses, and a lab coat at all times when conducting the procedure
- Carefully performing all procedures to minimize the creation of aerosols or splashes

Required Media for RAGE/HEK293 Cell Line

The list below shows the recommended complete medium and freezing medium for maintenance of the RAGE/HEK293 Cell Line.

- D-MEM, high glucose
- 10 % fetal bovine serum (FBS)
- 2 mM L-glutamine
- 1 % Penicillin/Streptomycin (10,000 I.U. Penicillin and 10,000 µg/ml Streptomycin)
- 200 µg/ml of G418

Thawing Cells

Use the following protocol to thaw the RAGE/HEK293 Cell Line to initiate the culture. The initial propagation of cells should be used to generate stocks to be frozen and stored for future use.

1. Remove the frozen vial of cells from liquid nitrogen and quickly thaw them by swirling in a 37°C water bath. Try to keep the O-ring and cap of the vial out of the water, to prevent possible contamination. Wear eye protection.

2. Before the cells are completely thawed, remove from 37°C water bath and decontaminate outside of the vial with 70 % ethanol.

3. Using sterile techniques, transfer the cells to a T-75 cm^2 tissue culture flask containing 15 ml of complete medium at room temperature. Transfer entire contents of the vial to the T-75 flask, and do not pipette cells up and down as this may kill the cells.

4. Swirl the T-75 flask to evenly distribute cells. Incubate the flask at 37°C, 5 % CO_2 overnight to allow cells to attach to the bottom of the flask.

5. The following day, pour off or aspirate off medium and replace with 15 ml fresh complete medium at room temperature.

6. Incubate the cells at 37°C, 5 % CO_2 and check daily until they reach 85-95 % confluency (about 2-4 days).

7. Once the cells reach 85-95 % confluency, subculture the cells as described below. For the initial culture, it is recommended to archive several frozen stocks and continue to propagate remainder of cells for use in experiments.

Note: Vials inappropriately stored directly in liquid nitrogen without protective tubing, such as Nunc

Cryoflex™ Tubing, may contain liquid nitrogen. Upon thawing, the liquid nitrogen will quickly convert to the gas phase and may cause the vial of cells to explode. This is a very hazardous situation, and should only be performed using protective gloves and clothing, and a full-face mask. To avoid this situation, store vials only in the vapor phase of liquid nitrogen or use the protective tubing described above if the vial must be stored directly in the liquid phase of liquid nitrogen.

Subculturing Cells

When the cells reach 85-95 % confluency, they are ready to be subcultured, or transferred to a new tissue culture flask. This is typically every 2-3 days. Use the following protocol to subculture the cells grown in a T-75 cm^2 flask. If a different sized tissue culture flask is being used, scale the reagent and media volumes accordingly.

1. Remove complete medium from the flask by pouring or aspiration. Wash the cells once with 5 ml PBS to remove excess medium, and discard PBS. Complete medium containing FBS will inhibit trypsin.

2. Add 5 ml of pre-warmed (room temperature to 37°C) trypsin-EDTA (0.5 % trypsin with EDTA-2Na) solution to the cell monolayer and incubate for 5 minutes at 37°C, 5 % CO_2, or until cells detach. If cells are still attached after 5 minutes, swirl the flask gently and incubate a few minutes longer.

3. Add 5 ml of complete medium and gently pipette up and down to break up cell clumps and achieve a suspension of single cells. Transfer the cell suspension to a 15 ml sterile, conical centrifuge tube.

4. Determine viable and total cell counts by use of a hemocytometer chamber or a Coulter Counter.

5. Dispense 1 ml of the cell suspension into each new T-75 cm^2 flask containing 20 ml of pre-warmed medium. This is a 1:10 split (1/10) of the original cell population. Cells should be 85-95 % confluent after 2 to 3 days. If using a culture flask other than a T-75 cm^2, scale the volume of cell suspension used. If cells are to be used for an experimental assay, seed cells at the required density for the experiment.

6. Incubate the cells at 37°C, 5 % CO_2 until 85-95 % confluent and subculture again, or incubate until they reach the desired confluency for the experiment.

Preparation of frozen cell stocks

Before beginning the freezing protocol below, label all cryovials and prepare freezing medium (complete growth medium with 10 % DMSO). Keep freezing medium at 4°C or on ice until ready for use.

1. Culture a T-75 cm^2 flask of the cells to 85-95 % confluency.

2. Remove the cells from the flask by following steps 1 through 5 in "Subculturing RAGE/HEK293 Cell Line", above.

3. Centrifuge the remaining cell suspension at 250 × g for 10 minutes at room temperature. Aspirate the medium from the cells and resuspend the pelleted cells in 1 ml of freezing medium for every 1 ml of original cell suspension (e.g., if the cells retrieved from the original T-75 cm^2 flask are resuspended in 10 ml and 1 ml is used for subculturing, centrifuge the remaining 9 ml of cells, aspirate medium, and

resuspend in 9 ml of freezing medium. If more or less cell suspension is used, adjust the volume of freezing medium accordingly). Each T-75 cm^2 flask at 85-95 % confluency will yield approximately ten (10) of 1 ml aliquot for freezing.

4. Dispense the 1 ml aliquots of the cells into cryovials following manufacturer's recommendations.

5. Freeze cells using either a controlled-rate freezing apparatus or manually using a freezing container. The apparatus should provide a controlled freezing rate of 1°C/minute. Cells should be frozen to -70°C to -80°C overnight.

6. Transfer frozen cell stocks to liquid nitrogen storage the following day.

Character of RAGE/HEK293 Cell Line

Fig.1 Western blotting of RAGE/HEK293 Cell Line by anti-His-tag monoclonal antibody

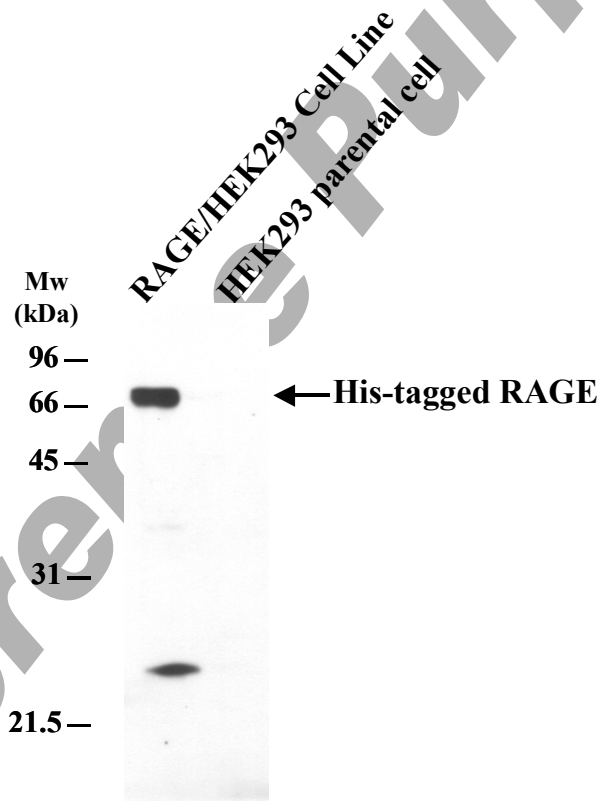
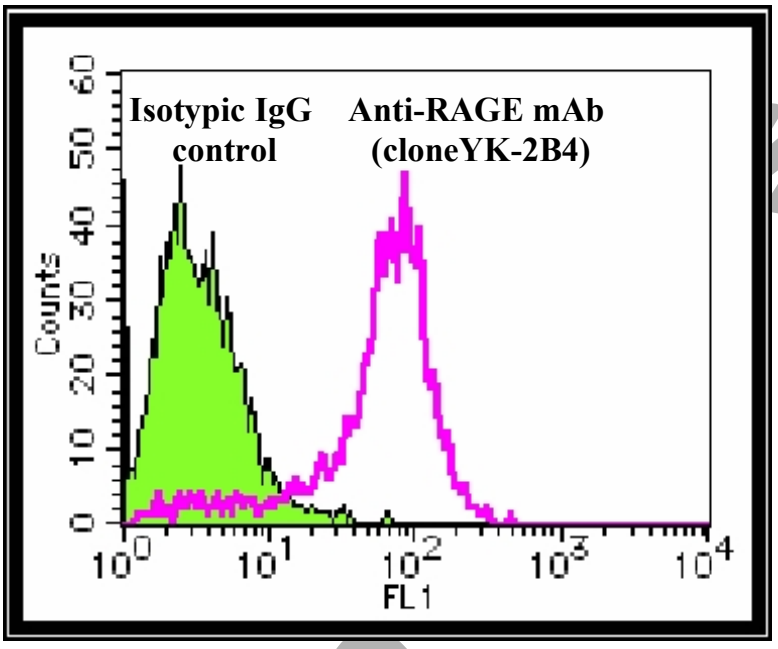


Fig.2 Flow cytometory analysis of RAGE/HEK293 Cell Line by anti-RAGE monoclonal antibody, YK-2B4 (CY-M1038)



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Related Products

*** Anti-RAGE monoclonal antibody: Cat# CY-M1038**
*** CD36/HEK293 cell line: Cat# CY-C8251**
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*** Glucose-AGE-BSA: CY-R2056**
*** Glucose-AGE-OVA: CY-R2057**
*** Glyceraldehyde-AGE-BSA: Cat# CY-R2058**
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*** Human S100B: Cat# CY-R2250**
*** Human S100A6: Cat# CY-R2256**
*** Human S100A12: Cat# CY-R2262G**
*** Human S100A12: Cat# CY-R2262H**
*** Human S100P: Cat# CY-R2267**

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