SANTA CRUZ BIOTECHNOLOGY, INC.

CRTH2 siRNA (m): sc-77327

BACKGROUND

The human chemoattractant receptor-homologous molecule (CRTH2, GPR44, G protein-coupled receptor 44) maps to chromosome 11q12.2 and encodes a 472 amino acid G protein-coupled leukocyte chemoattractant receptor. Chemoattractant receptors present on Th2 cells respond to parasites and play a central role in allergic inflammation; they are absent on type 1 T helper (Th1) cells, which address intracellular bacteria and many viruses. CRTH2 contains seven putative transmembrane domains and mediates signals to the interior of the cell upon exposure to its cognate ligand prostaglandin (PG) D2, which is able to attract basophils, eosinophils, type 2 Th (Th2) cells and type 2 cytotoxic (Tc2) $CD8^+$ T lymphocytes. CRTH2 expression on active Th2 cells influences supportive roles in Th2-type immune reactions. 3.5 kb CRTH2 transcripts are present in thalamus, frontal cortex, pons, hippocampus, hypothalamus and caudate, while 3.4 kb transcripts are present in fetal liver, leukocytes and thymus.

REFERENCES

1. Marchese, A., et al. 1999. Discovery of three novel orphan G protein-coupled receptors. Genomics 56: 12-21.
2. Cosmi, L., et al. 2000. CRTH2 is the most reliable marker for the detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. Eur. J. Immunol. 30: 2972-2979.
3. Cosmi, L., et al. 2001. Chemoattractant receptors expressed on type 2 T cells and their role in disease. Int. Arch. Allergy Immunol. 125: 273-279.
4. Cosmi, L., et al. 2001. CRTH2: marker for the detection of human Th2 and Tc2 cells. Adv. Exp. Med. Biol. 495: 25-29.
5. Annunziato, F., et al. 2001. Reversal of human allergen-specific $CRTH2^+$ Th2 cells by IL-12 or the PS-DSP30 oligodeoxynucleotide. J. Allergy Clin. Immunol. 108: 815-821.
6. Michimata, T., et al. 2002. Accumulation of CRTH2-positive T-helper 2 and T-cytotoxic 2 cells at implantation sites of human decidua in a prostaglandin D2-mediated manner. Mol. Hum. Reprod. 8: 181-187.
7. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 604837. World Wide Web URL: http://www.ncbi.nlm. nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Gpr44 (mouse) mapping to 19 A.

PRODUCT

CRTH2 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CRTH2 shRNA Plasmid (m): sc-77327-SH and CRTH2 shRNA (m) Lentiviral Particles: sc-77327-V as alternate gene silencing products.

For independent verification of CRTH2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-77327A, sc-77327B and sc-77327C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CRTH2 siRNA (m) is recommended for the inhibition of CRTH2 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

CRTH2 (C-5): sc-271898 is recommended as a control antibody for monitoring of CRTH2 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CRTH2 gene expression knockdown using RT-PCR Primer: CRTH2 (m)-PR: sc-77327-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.