

## IL-3R $\alpha$ siRNA (m): sc-35660

### BACKGROUND

Interleukin-3, or IL-3, is a pleiotropic cytokine that is primarily secreted by activated T lymphocytes and stimulates the proliferation and differentiation of hematopoietic cells. IL-3 exerts its biological effects through a receptor which consists of a ligand-specific  $\alpha$  subunit (IL-3R $\alpha$ ) and a signal transducing  $\beta$  subunit (IL-3R $\beta$ ) common to the IL-3/IL-5/GM-CSF receptors. The  $\alpha$  subunits are low-affinity ligand-binding proteins while the  $\beta$  subunits do not themselves bind ligand, but are required for high affinity binding by the  $\alpha$  subunits. The mouse IL-3 receptor has two distinct  $\beta$  subunits, one that functions only in IL-3-mediated cell signaling and a second that is shared with IL-5 and GM-CSF. The murine  $\beta$  subunits are 91% homologous at the amino acid level but only 56% homologous to the human  $\beta$  subunit. The carboxy-terminus of the  $\beta$  subunit has been shown to be necessary for activation of the MAP kinase signaling pathway. Although the IL-3 receptor has no intrinsic kinase activity, stimulation with IL-3 leads to tyrosine phosphorylation of the JAK/Tyk 2 family member, JAK2, which in turn activates and causes nuclear translocation of Stat5a and Stat5b.

### REFERENCES

- Hayashida, K., et al. 1990. Molecular cloning of a second subunit of the receptor for human granulocyte macrophage colony-stimulating factor (GM-CSF): reconstitution of a high affinity GM-CSF receptor. *Proc. Natl. Acad. Sci. USA* 87: 9655-9659.
- Tavernier, J., et al. 1992. A human high affinity interleukin-5 receptor (IL-5R) is composed of an IL-5 specific chain and a  $\beta$  chain shared with the receptor for GM-CSF. *Cell* 66: 1175-1184.
- Hara, T., et al. 1992. Two distinct functional receptors for mouse interleukin-3. *EMBO J.* 11: 1875-1884.
- Sakamaki, K., et al. 1992. Critical cytoplasmic domains of the common  $\beta$  subunit of the human GM-CSF, IL-3, and IL-5 receptors for growth signal transduction and tyrosine phosphorylation. *EMBO J.* 11: 3541-3549.
- Park, L.S., et al. 1992. Cloning of the low-affinity murine granulocyte-macrophage colony-stimulating factor receptor and reconstitution of a high-affinity receptor complex. *Proc. Natl. Acad. Sci. USA* 89: 4295-4299.
- Miyajima, A., et al. 1992. Cytokine receptors and signal transduction. *Annu. Rev. Immunol.* 10: 295-331.

### CHROMOSOMAL LOCATION

Genetic locus: Il3ra (mouse) mapping to 14 A2.

### PRODUCT

IL-3R $\alpha$  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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IL-3R $\alpha$  shRNA Plasmid (m): sc-35660-SH and IL-3R $\alpha$  shRNA (m) Lentiviral Particles: sc-35660-V as alternate gene silencing products.

For independent verification of IL-3R $\alpha$  (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-35660A, sc-35660B and sc-35660C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

IL-3R $\alpha$  siRNA (m) is recommended for the inhibition of IL-3R $\alpha$  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  $\mu$ M in 66  $\mu$ l. 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

IL-3R $\alpha$  (C-5): sc-74522 is recommended as a control antibody for monitoring of IL-3R $\alpha$  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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IL-3R $\alpha$  gene expression knockdown using RT-PCR Primer: IL-3R $\alpha$  (m)-PR: sc-35660-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.