## BACKGROUND

The c-Kit proto-oncogene is a member of the receptor tyrosine kinase family and, more specifically, is closely related to the platelet derived growth factor receptor (PDGFR). c-Kit, the normal cellular homolog of the HZ4-feline sarcoma virus transforming gene (v-Kit), encodes a transmembrane receptor. c-Kit regulates a variety of biological responses including chemotaxis, cell proliferation, apoptosis and adhesion. c-Kit is also identical with the product of the W locus in mice and, as such, is integral to the development of Mast cells and hematopoiesis. The ligand for the c-Kit receptor (KL) has been identified and is encoded at the murine steel (SI) locus. Kit is the human homolog of the proto-oncogene c-Kit. Mutations in Kit are integral for tumor growth and progression in various cancers.

## REFERENCES

1. Besmer, P., et al. 1986. A new acute transforming feline retrovirus and relationship of its oncogene $v$-Kit with the protein kinase gene family. Nature 320: 415-417.
2. Yarden, Y., et al. 1987. Human proto-oncogene c-Kit: a new cell surface receptor kinase for an unidentified ligand. EMBO J. 6: 3341-3347.
3. Majumder, S., et al. 1988. c-Kit protein, a transmembrane kinase: identification in tissues and characterization. Mol. Cell. Biol. 8: 4896-5002.
4. Chabot, B., et al. 1988. The proto-oncogene c-Kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature 335: 88-90.
5. Geissler, E.N., et al. 1988. The dominant-white spotting (W) locus of the mouse encodes the c-Kit proto-oncogene. Cell 55: 185-195.
6. Flanagan, J.G., et al. 1990. The Kit ligand: a cell surface molecule altered in steel mutant fibroblasts. Cell 63: 185-194.
7. Lerner, N.B., et al. 1991. Monoclonal antibody YB5.B8 identifies the human c-Kit protein product. Blood 77: 1876-1883.

## CHROMOSOMAL LOCATION

Genetic locus: Kit (rat) mapping to 14p11.

## PRODUCT

c-Kit siRNA (r) 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 $10 \mu \mathrm{M}$ solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see c-Kit shRNA Plasmid (r): sc-63363-SH and c-Kit shRNA (r) Lentiviral Particles: sc-63363-V as alternate gene silencing products.
For independent verification of c -Kit (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63363A, sc-63363B and sc-63363C.

## PROTOCOLS

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

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at $-20^{\circ} \mathrm{C}$ with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at $-20^{\circ} \mathrm{C}$, avoid contact with RNAses and repeated freeze thaw cycles.
Resuspend lyophilized siRNA duplex in $330 \mu \mathrm{l}$ of the RNAse-free water provided. Resuspension of the siRNA duplex in $330 \mu$ l of RNAse-free water makes a $10 \mu \mathrm{M}$ solution in a $10 \mu \mathrm{M}$ Tris- $\mathrm{HCl}, \mathrm{pH} 8.0,20 \mathrm{mM} \mathrm{NaCl}, 1 \mathrm{mM}$ EDTA buffered solution.

## APPLICATIONS

c -Kit siRNA (r) is recommended for the inhibition of c-Kit expression in rat 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 $10 \mu \mathrm{M}$ in $66 \mu \mathrm{l}$. 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

c-Kit (E-3): sc-365504 is recommended as a control antibody for monitoring of c-Kit 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-IgGк BP-HRP: sc-516102 or m-IgGк BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz ${ }^{\circledR}$ Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgGк BP-FITC: sc-516140 or m-lgGк BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz ${ }^{\circledR}$ Mounting Medium: sc-24941 or UltraCruz ${ }^{\circledR}$ Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor c-Kit gene expression knockdown using RT-PCR Primer: c-Kit (r)-PR: sc-63363-PR ( $20 \mu \mathrm{l}, 445 \mathrm{bp}$ ). Annealing temperature for the primers should be $55-60^{\circ} \mathrm{C}$ and the extension temperature should be $68-72^{\circ} \mathrm{C}$.

## RESEARCH USE

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

