SANTA CRUZ BIOTECHNOLOGY, INC.

Brk siRNA (m): sc-38940

BACKGROUND

Tyrosine protein kinases play crucial roles in cell proliferation, survival, adhesion and motility by regulating ligand-mediated signal transduction, cell-cycle progression and cytoskeleton function. Tyrosine kinases may also bring about the transformation of malignant cells. Breast tumor kinase, Brk (also known as PTK6), along with its murine homolog, Sik (src-related intestinal kinase) is one such kinase. Brk is a member of a distinct family of intracellular tyrosine kinases thought to be related to the Src family of tumor-related kinases. Brk exhibits the features of a novel non-receptor tyrosine kinase, including N-terminal SH3 and SH2 domains. Brk is specifically expressed in epithelial tissues and is restricted to cell layers immediately above the proliferative cell zone in skin and alimentary canal lining. Expression of Brk in normal tissues is relatively restricted with the highest mRNA levels found in colon, small intestine and prostate. Brk is strongly expressed in many breast carcinomas but not in normal breast tissue. Brk protein is also capable of autophosphorylation, which may play a role in its regulation.

REFERENCES

1. Wilks, A.F. 1989. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc. Natl. Acad. Sci. USA 86: 1603-1607.
2. Lee, S.T., et al. 1993. A survey of protein tyrosine kinase mRNAs expressed in normal human melanocytes. Oncogene 8: 3403-3410.
3. Siyanova, E.Y., et al. 1994. Tyrosine kinase gene expression in the mouse small intestine. Oncogene 9: 2053-2057.
4. Mitchell, P.J., et al. 1994. Cloning and characterization of cDNAs encoding a novel non-receptor tyrosine kinase, Brk, expressed in human breast tumours. Oncogene 9: 2383-2390.
5. Vasioukhin V., et al. 1995. A novel intracellular epithelial cell tyrosine kinase is expressed in the skin and gastrointestinal tract. Oncogene 10: 349-357.
6. Qiu, H. and Miller, W.T. 2002. Regulation of the nonreceptor tyrosine kinase Brk by autophosphorylation and by autoinhibition. J. Biol. Chem. 277: 34634-34641.

CHROMOSOMAL LOCATION

Genetic locus: Ptk6 (mouse) mapping to 2 H4.

PRODUCT

Brk 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 Brk shRNA Plasmid (m): sc-38940-SH and Brk shRNA (m) Lentiviral Particles: sc-38940-V as alternate gene silencing products.

For independent verification of Brk (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-38940A, sc-38940B and sc-38940C.

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

Brk siRNA (m) is recommended for the inhibition of Brk 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

Brk (G-6): sc-166171 is recommended as a control antibody for monitoring of Brk 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 Brk gene expression knockdown using RT-PCR Primer: Brk (m)-PR: sc-38940-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.