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c-Abl shRNA (h2) Lentiviral Particles: sc-44290-V

BACKGROUND

The Abl oncogene was initially identified as the viral transforming gene of Abelson murine leukemia virus (A-MuLV). The major translational product of c-Abl has been identified as a protein with tyrosine kinase activity and an SH2 domain. The Abl oncogene is implicated in several human leukemias including 90-95% of chronic myelocytic leukemia (CML), 20-25% of adult acute lymphoblastic leukemia (ALL) and 2-5% of pediatric ALL. In these leukemias the c-Abl proto-oncogene undergoes a chromosomal translocation producing the Philadelphia (Ph1) chromosome. The molecular consequence of this translocation is the generation of a chimeric Bcr/c-Abl mRNA encoding activated Abl protein-tyrosine kinase. The Bcr gene has been shown to encode a GTPase-activating protein (GAP) specific for the Ras-related GTP-binding protein, p21Rac.

REFERENCES

1. Abelson, H.T., et al. 1970. Lymphosarcoma: virus-induced thymic-independent disease in mice. *Cancer Res.* 30: 2213-2222.
2. de Klein, A., et al. 1982. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukemia. *Nature* 300: 765-767.
3. Prywes, R., et al. 1983. Sequences of the A-MuLV protein needed for fibroblasts and lymphoid cell transformation. *Cell* 34: 569-579.
4. Konopka, J.B., et al. 1984. An alteration of the human c-Abl protein in K-562 leukemia cells unmasks associated tyrosine kinase activity. *Cell* 37: 1035-1042.
5. Stam, K., et al. 1985. Evidence of a new chimeric Bcr/c-Abl mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. *N. Engl. J. Med.* 313: 1429-1433.
6. Diekmann, D., et al. 1991. Bcr encodes a GTPase-activating protein for p21Rac. *Nature* 351: 400-402.
7. Overduin, M., et al. 1992. Three-dimensional solution structure of the Src homology 2 domain of c-Abl. *Cell* 70: 697-704.

CHROMOSOMAL LOCATION

Genetic locus: ABL1 (human) mapping to 9q34.12.

PRODUCT

c-Abl shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see c-Abl siRNA (h2): sc-44290 and c-Abl shRNA Plasmid (h2): sc-44290-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

c-Abl shRNA (h2) Lentiviral Particles is recommended for the inhibition of c-Abl expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

c-Abl (K-12) : sc-131 is recommended as a control antibody for monitoring of c-Abl 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor c-Abl gene expression knockdown using RT-PCR Primer: c-Abl (h2)-PR: sc-44290-PR (20 µl, 508 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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