

PLZF siRNA (r): sc-156168

BACKGROUND

Hypermethylated in cancer (HIC-1) was originally identified as a target of p53-induced gene expression. HIC-1 is deleted in the genetic disorder Miller-Dieker syndrome (MDS). The expression of HIC-1 is also frequently suppressed in leukemia and other various cancers due to the hypermethylation of specific DNA regions and the resulting transcriptional silencing. These and other studies indicate that HIC-1 acts as a putative tumor suppressor protein that mediates transcriptional repression. HIC-1 is ubiquitously expressed in adult tissues and its structure is defined by five zinc fingers and an N-terminal broad-complex POZ (or BTB) domain. The BTB/POZ domain mediates homomeric and heteromeric POZ-POZ interactions and is common to transcriptional regulators involved in chromatin modeling. In several BTB/POZ containing proteins, including BCL-6 and the promyelocytic leukemia zinc finger (PLZF) oncoprotein, this domain interacts with the SMRT/N-CoR-mSin3A HDAC complex and is directly involved in repressing and silencing gene transcription. When this domain is deleted, as with the oncogenic PLZF-RAR chimera of promyelocytic leukemias, this transcriptional repression is attenuated. Conversely, HIC-1 does not interact with components of the HDAC complex, suggesting that HIC-1-induced transcriptional repression is unassociated with the POZ/BTB domain.

REFERENCES

1. Wales, M.M., et al. 1995. p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. *Nat. Med.* 1: 570-577.
2. Ahmad, K.F., et al. 1998. Crystal structure of the BTB domain from PLZF. *Proc. Natl. Acad. Sci. USA* 95: 12123-12128.
3. David, G., et al. 1998. Histone deacetylase associated with mSin3A mediates repression by the acute promyelocytic leukemia-associated PLZF protein. *Oncogene* 16: 2549-2556.
4. Huynh, K.D., et al. 1998. The Bcl-6 POZ domain and other POZ domains interact with the corepressors N-CoR and SMRT. *Oncogene* 17: 2473-2484.
5. Wong, C.W., et al. 1998. Components of the SMRT corepressor complex exhibit distinctive interactions with the POZ domain oncoproteins PLZF, PLZF-RAR α , and Bcl-6. *J. Biol. Chem.* 273: 27695-27702.

CHROMOSOMAL LOCATION

Genetic locus: Zbtb16 (rat) mapping to 8q23.

PRODUCT

PLZF siRNA (r) is a pool of 2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PLZF shRNA Plasmid (r): sc-156168-SH and PLZF shRNA (r) Lentiviral Particles: sc-156168-V as alternate gene silencing products.

For independent verification of PLZF (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-156168A and sc-156168B.

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

APPLICATIONS

PLZF siRNA (r) is recommended for the inhibition of PLZF 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 μ M in 66 μ 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

PLZF (D-9): sc-28319 is recommended as a control antibody for monitoring of PLZF 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PLZF gene expression knockdown using RT-PCR Primer: PLZF (r)-PR: sc-156168-PR (20 μ 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 for detailed protocols and support products.