

Chfr siRNA (h): sc-37567

BACKGROUND

The forkhead-associated (FHA) domain was initially identified in transcription factors that have forkhead DNA-binding domains and in protein kinases, but many cell-cycle checkpoint proteins, including Chfr (checkpoint with forkhead and RING finger domains) contain FHA domains. Chfr defines a checkpoint that delays entry into metaphase in response to mitotic stress. Normal primary cells and tumor cell lines that express wild-type Chfr exhibit delayed entry into metaphase when centrosome separation is inhibited by mitotic stress. Additionally, Chfr seems to be required for delaying prophase in human cells. The sequence of Chfr is similar to that of the fission yeast DMA1, which is involved in a later mitotic checkpoint that delays a cell's exit from mitosis in response to spindle damage.

REFERENCES

1. Jha, M.N., et al. 1994. Cell cycle arrest by Colcemid differs in human normal and tumor cells. *Cancer Res.* 54: 5011-5015.
2. Hofmann, K., et al. 1995. The FHA domain: a putative nuclear signalling domain found in protein kinases and transcription factors. *Trends Biochem. Sci.* 20: 347-349.
3. Murone, M., et al. 1996. The fission yeast dma1 gene is a component of the spindle assembly checkpoint, required to prevent septum formation and premature exit from mitosis if spindle function is compromised. *EMBO J.* 15: 6605-6616.
4. Cortez, D., et al. 2000. Conducting the mitotic symphony. *Nature* 406: 354-356.

CHROMOSOMAL LOCATION

Genetic locus: CHFR (human) mapping to 12q24.33.

PRODUCT

Chfr siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Chfr shRNA Plasmid (h): sc-37567-SH and Chfr shRNA (h) Lentiviral Particles: sc-37567-V as alternate gene silencing products.

For independent verification of Chfr (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37567A, sc-37567B and sc-37567C.

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

Chfr siRNA (h) is recommended for the inhibition of Chfr expression in human 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

Chfr (L2): sc-81832 is recommended as a control antibody for monitoring of Chfr 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 Chfr gene expression knockdown using RT-PCR Primer: Chfr (h)-PR: sc-37567-PR (20 μ l, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kannan, S., et al. 2019. Anti-leukemia effects of Notch-mediated inhibition of oncogenic PLK1 in B-cell acute lymphoblastic leukemia. *Mol. Cancer Ther.* 18: 1615-1627.

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.