



FANCF siRNA (h): sc-40570

BACKGROUND

Fanconi anemia (FA) is an autosomal recessive disorder characterized by bone marrow failure, birth defects and chromosomal instability. At the cellular level, FA is characterized by spontaneous chromosomal breakage and a unique hypersensitivity to DNA cross-linking agents. At least eight complementation groups (A-G) have been identified and six FA genes (for subtypes A, C, D2, E, F and G) have been cloned. The FA proteins lack sequence homologies or motifs that could point to a molecular function. Phosphorylation of FANCF (Fanconi anemia complementation group) proteins is thought to be important for the function of the FA pathway. FA proteins are encoded by six cloned FA genes (FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG) and cooperate in a common pathway, culminating in the monoubiquitination of FANCD2 protein and colocalization of FANCD2 and BRCA1 proteins in nuclear foci. FANCF protein is required for FANCD2 activation and appears to stabilize other subunits of the complex. The human FANCF gene maps to chromosome 11p15 and encodes a nuclear protein with homology to the prokaryotic RNA-binding protein ROM.

REFERENCES

1. de Winter, J.P., et al. 2000. The Fanconi anemia protein FANCF forms a nuclear complex with FANCA, FANCC and FANCG. *Hum. Mol. Genet.* 9: 2665-2674.
2. Yagasaki, H., et al. 2001. A cytoplasmic serine protein kinase binds and may regulate the Fanconi anemia protein FANCA. *Blood* 98: 3650-3657.
3. Wilson, J.B., et al. 2001. The Chinese hamster FANCG/XRCC9 mutant NM3 fails to express the monoubiquitinated form of the FANCD2 protein, is hypersensitive to a range of DNA damaging agents and exhibits a normal level of spontaneous sister chromatid exchange. *Carcinogenesis* 22: 1939-1946.
4. Otsuki, T., et al. 2002. Phosphorylation of Fanconi anemia protein, FANCA, is regulated by Akt kinase. *Biochem. Biophys. Res. Commun.* 291: 628-634.

CHROMOSOMAL LOCATION

Genetic locus: FANCF (human) mapping to 11p15.

PRODUCT

FANCF siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FANCF shRNA Plasmid (h): sc-40570-SH and FANCF shRNA (h) Lentiviral Particles: sc-40570-V as alternate gene silencing products.

For independent verification of FANCF (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-40570A, sc-40570B and sc-40570C.

PROTOCOLS

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

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

FANCF siRNA (h) is recommended for the inhibition of FANCF 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 60 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

FANCF (N-20): sc-19327 is recommended as a control antibody for monitoring of FANCF 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FANCF gene expression knockdown using RT-PCR Primer: FANCF (h)-PR: sc-40570-PR (20 μ l, 495 bp). 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.