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FANCL siRNA (m): sc-45662

BACKGROUND

Defects in FANCL are a cause of Fanconi anemia. 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 8 complementation groups have been identified and 6 FA genes (for subtypes A, C, D2, E, F and G) have been cloned. Phosphorylation of FANCL (Fanconi anemia complementation group) proteins is thought to be important for the function of the FA pathway. FA proteins cooperate in a common pathway, culminating in the monoubiquitination of FANCD2 protein and colocalization of FANCD2 and BRCA1 proteins in nuclear foci. FANCL is a ligase protein mediating the ubiquitination of FANCD2, a key step in the DNA damage pathway. FANCL may be required for proper primordial germ cell proliferation in the embryonic stage.

REFERENCES

1. Meetei, A.R., et al. 2003. A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat. Genet.* 35: 165-1670.
2. Kutler, D.I., et al. 2004. Fanconi anemia in Ashkenazi Jews. *Fam. Cancer* 3: 241-248.
3. Meetei, A.R., et al. 2004. X-linked inheritance of Fanconi anemia complementation group B. *Nat. Genet.* 36: 1219-1224.
4. Fei, P., et al. 2005. New advances in the DNA damage response network of Fanconi anemia and BRCA proteins. FAAP95 replaces BRCA2 as the true FANCL protein. *Cell Cycle* 4: 80-86.
5. Meetei, A.R., et al. 2005. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat. Genet.* 37: 958-963.

CHROMOSOMAL LOCATION

Genetic locus: Fancl (mouse) mapping to 11 A3.3.

PRODUCT

FANCL 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FANCL shRNA Plasmid (m): sc-45662-SH and FANCL shRNA (m) Lentiviral Particles: sc-45662-V as alternate gene silencing products.

For independent verification of FANCL (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-45662A, sc-45662B and sc-45662C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

FANCL shRNA (m) Lentiviral Particles is recommended for the inhibition of FANCL 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 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

FANCL (C-4): sc-137076 is recommended as a control antibody for monitoring of FANCL 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FANCL gene expression knockdown using RT-PCR Primer: FANCL (m)-PR: sc-45662-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.