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FANCL shRNA (m) Lentiviral Particles: sc-45662-V

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 FANCD2 (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 FANCB 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 shRNA (m) 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 μ l 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 FANCL siRNA (m): sc-45662 and FANCL shRNA Plasmid (m): sc-45662-SH as alternate gene silencing products.

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.

APPLICATIONS

FANCL shRNA (m) Lentiviral Particles is recommended for the inhibition of FANCL expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

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.

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.

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.

PROTOCOLS

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