SANTA CRUZ BIOTECHNOLOGY, INC.

FANCD2 siRNA (h): sc-35356

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 FANC (Fanconi anemia complementation group) proteins are thought to be important for the function of the FA pathway. Several FA proteins, including FANCA, FANCC, FANCF and FANCG, interact in a nuclear complex, and this complex is required for the activation (monoubiquitination) of the downstream FANCD2 protein. When monoubiquitinated, the FANCD2 protein co-localizes with the breast cancer susceptibility protein BRCA1 in DNA damage induced foci. In male meiosis, FANCD2 also co-localizes with BRCA1 at synaptonemal complexes. The human FANCD2 gene maps to chromosome 3p25.3, contains 44 exons and encodes a 1,451 amino acid nuclear protein that exists as 2 protein isoforms.

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. Grompe, M. and D'Andrea, A. 2001. Fanconi anemia and DNA repair. Hum. Mol. Genet. 10: 2253-2259.

CHROMOSOMAL LOCATION

Genetic locus: FANCD2 (human) mapping to 3p25.3.

PRODUCT

FANCD2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FANCD2 shRNA Plasmid (h): sc-35356-SH and FANCD2 shRNA (h) Lentiviral Particles: sc-35356-V as alternate gene silencing products.

For independent verification of FANCD2 (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-35356A, sc-35356B and sc-35356C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

FANCD2 siRNA (h) is recommended for the inhibition of FANCD2 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 µM in 66 µl. 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

FANCD2 (FI17): sc-20022 is recommended as a control antibody for monitoring of FANCD2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 FANCD2 gene expression knockdown using RT-PCR Primer: FANCD2 (h)-PR: sc-35356-PR (20 µl, 510 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kumari, U., et al. 2014. Evidence of mitochondrial dysfunction and impaired ROS detoxifying machinery in Fanconi anemia cells. Oncogene 33: 165-172.
2. Xia, P., et al. 2015. p53 mediated apoptosis in osteosarcoma MG-63 cells by inhibition of FANCD2 gene expression. Int. J. Clin. Exp. Med. 8: 11101-11108.
3. Miller, H.E., et al. 2020. Reconstruction of Ewing sarcoma developmental context from mass-scale transcriptomics reveals characteristics of EWSR1-FLI1 permissibility. Cancers 12: 948.

RESEARCH USE

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