

## FBL5 siRNA (h): sc-62302

### BACKGROUND

FBL5 is a 691 amino acid protein encoded by the human gene FBXL5. FBL5 contains one 40 amino acid F-box region, making it a member of the F-box family. FBL5 also contains four LRR (leucine-rich) repeats. F-box proteins are critical components of the SCF (skp1-CUL-1-F-box protein) type E3 ubiquitin ligase complex and are involved in substrate recognition and recruitment for ubiquitination. F-box proteins are members of a large family that regulates cell cycle, immune response, signalling cascades and developmental programs by targeting proteins, such as cyclins, cyclin-dependent kinase inhibitors, I $\kappa$ B- $\alpha$  and  $\beta$ -catenin, for degradation by the proteasome after ubiquitination. Localized near the nucleus in the cytoplasm, FBL5 is ubiquitously expressed and believed to recognize and bind to phosphorylated proteins to promote their ubiquitination and degradation.

### REFERENCES

1. Winston, J.T., et al. 1999. The SCF <sup>$\beta$ -TrCP</sup>-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in I $\kappa$ B $\alpha$  and  $\beta$ -catenin and stimulates I $\kappa$ B $\alpha$  ubiquitination *in vitro*. *Genes Dev.* 13: 270-283.
2. Cenciarelli, C., et al. 1999. Identification of a family of human F-box proteins. *Curr. Biol.* 9: 1177-1179.
3. Winston, J.T., et al. 1999. A family of mammalian F-box proteins. *Curr. Biol.* 9: 1180-1182.
4. Craig, K.L. and Tyers, M. 1999. The F-box: a new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. *Prog. Biophys. Mol. Biol.* 72: 299-328.
5. Ilyin, G.P., et al. 2000. cDNA cloning and expression analysis of new members of the mammalian F-box protein family. *Genomics* 67: 40-47.
6. Schulman, B.A., et al. 2000. Insights into SCF ubiquitin ligases from the structure of the Skp1-Skp2 complex. *Nature* 408: 381-386.
7. Ilyin, G.P., et al. 2002. A new subfamily of structurally related human F-box proteins. *Gene* 296: 11-20.

### CHROMOSOMAL LOCATION

Genetic locus: FBXL5 (human) mapping to 4p15.32.

### PRODUCT

FBL5 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FBL5 shRNA Plasmid (h): sc-62302-SH and FBL5 shRNA (h) Lentiviral Particles: sc-62302-V as alternate gene silencing products.

For independent verification of FBL5 (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-62302A, sc-62302B and sc-62302C.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

FBL5 siRNA (h) is recommended for the inhibition of FBL5 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  $\mu$ M in 66  $\mu$ 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

FBL5 (G-11): sc-390102 is recommended as a control antibody for monitoring of FBL5 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FBL5 gene expression knockdown using RT-PCR Primer: FBL5 (h)-PR: sc-62302-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Azmi, A.S., et al. 2015. Targeting the nuclear export protein XPO1/CRM1 reverses epithelial to mesenchymal transition. *Sci. Rep.* 5: 16077.

### RESEARCH USE

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