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TRABID siRNA (m): sc-154577

BACKGROUND

TRABID (TRAF-binding domain-containing protein), also known as ZRANB1 (zinc finger Ran-binding domain-containing protein 1), is a 708 amino acid cytoplasmic and nuclear protein that is widely expressed. Belonging to the peptidase C64 family, TRABID is considered a positive regulator of the Wnt signaling pathway that specifically cleaves "Lys-63"-linked ubiquitin chains. TRABID acts by deubiquitinating APC, a negative regulator of Wnt-mediated transcription. TRABID contains a OTU domain, which mediates the deubiquitinating activity, and three RanBP2-type zinc fingers that mediate the specific interaction with "Lys-63"-linked ubiquitin. It is suggested that TRABID may also modulate TNF α signaling. The gene encoding TRABID is located on chromosome 10, which houses over 1,200 genes and comprises nearly 4.5% of the human genome.

REFERENCES

- Evans, P.C., et al. 2001. Isolation and characterization of two novel A20-like proteins. *Biochem. J.* 357: 617-623.
- Deloukas, P., et al. 2004. The DNA sequence and comparative analysis of human chromosome 10. *Nature* 429: 375-381.
- Komander, D. and Barford, D. 2008. Structure of the A20 OTU domain and mechanistic insights into deubiquitination. *Biochem. J.* 409: 77-85.
- Tran, H., et al. 2008. Trabad, a new positive regulator of Wnt-induced transcription with preference for binding and cleaving K63-linked ubiquitin chains. *Genes Dev.* 22: 528-542.
- Online Mendelian Inheritance in Man, OMIM[™]. 2008. Johns Hopkins University, Baltimore, MD. MIM Number: 611749. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Zranb1 (mouse) mapping to 7 F3.

PRODUCT

TRABID 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 TRABID shRNA Plasmid (m): sc-154577-SH and TRABID shRNA (m) Lentiviral Particles: sc-154577-V as alternate gene silencing products.

For independent verification of TRABID (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-154577A, sc-154577B and sc-154577C.

RESEARCH USE

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

PROTOCOLS

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

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

TRABID siRNA (m) is recommended for the inhibition of TRABID 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

TRABID (F-1): sc-374377 is recommended as a control antibody for monitoring of TRABID 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 TRABID gene expression knockdown using RT-PCR Primer: TRABID (m)-PR: sc-154577-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.