

OTUB1 shRNA Plasmid (m): sc-76015-SH

BACKGROUND

OTUB1 (OTU domain, ubiquitin aldehyde binding 1), also known as OTB1, OTU1, HSPC263 or Otubain-1, is a 271 amino acid protein that contains one OTU (ovarian tumor) domain and belongs to the OTU family of predicted cysteine proteases. Expressed as two isoforms (one of which is present throughout the body and the other of which is present only in lymphoid tissues), OTUB1 functions as a hydrolase that can remove ubiquitin residues from target proteins, thereby preventing protein degradation and playing an important role in protein turnover. OTUB1 interacts with GRAIL and, via this interaction, plays a role in the regulation and the induction of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to their cognate antigens. Due to its interaction with GRAIL, OTUB1 is an important regulator of immune responses in secondary lymphoid organs.

REFERENCES

1. Borodovsky, A., et al. 2002. Chemistry-based functional proteomics reveals novel members of the deubiquitinating enzyme family. *Chem. Biol.* 9: 1149-1159.
2. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608337. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Balakirev, M.Y., et al. 2003. Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Rep.* 4: 517-522.
4. Soares, L., et al. 2004. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. *Nat. Immunol.* 5: 45-54.
5. Juris, S.J., et al. 2006. Identification of otubain 1 as a novel substrate for the Yersinia protein kinase using chemical genetics and mass spectrometry. *FEBS Lett.* 580: 179-183.
6. Wang, M., et al. 2008. Comparative analysis of transcriptional profiling of CD3⁺, CD4⁺ and CD8⁺ T cells identifies novel immune response players in T-cell activation. *BMC Genomics* 9: 225.
7. Shan, T.L., et al. 2008. Partial molecular cloning, characterization, and analysis of the subcellular localization and expression patterns of the porcine OTUB1 gene. *Mol. Biol. Rep.* 36: 1573-1577.

CHROMOSOMAL LOCATION

Genetic locus: Otub1 (mouse) mapping to 19 A.

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.

PRODUCT

OTUB1 shRNA Plasmid (m) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see OTUB1 siRNA (m): sc-76015 and OTUB1 shRNA (m) Lentiviral Particles: sc-76015-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

OTUB1 shRNA Plasmid (m) is recommended for the inhibition of OTUB1 expression in mouse cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

OTUB1 (J-61): sc-130458 is recommended as a control antibody for monitoring of OTUB1 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 OTUB1 gene expression knockdown using RT-PCR Primer: OTUB1 (m)-PR: sc-76015-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.