SANTA CRUZ BIOTECHNOLOGY, INC.

OTUB1 siRNA (m): sc-76015

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.

PRODUCT

OTUB1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see OTUB1 shRNA Plasmid (m): sc-76015-SH and OTUB1 shRNA (m) Lentiviral Particles: sc-76015-V as alternate gene silencing products.

For independent verification of OTUB1 (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-76015A, sc-76015B and sc-76015C.

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

OTUB1 siRNA (m) is recommended for the inhibition of OTUB1 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 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

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.

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.