

USP45 siRNA (h): sc-76859

BACKGROUND

The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. A wide range of enzymes facilitate the proteolytic Ub pathway, including USPs (ubiquitin specific peptidases), which are cysteine proteases that are responsible for the release of ubiquitin from a ubiquitylated substrate and precursor fusion proteins. USP45 (ubiquitin carboxyl-terminal hydrolase 45), also known as deubiquitinating enzyme 45, is a 819 amino acid protein that is involved in ubiquitin-dependent protein catabolism. USP45 differs from other USPs in that it contains a UBP-type zinc finger, a domain which binds ubiquitin. Although USP45 is broadly expressed, the highest levels can be found in skeletal muscle, spleen and ovary. There are three isoforms of USP45 which are produced as a result of alternative splicing.

REFERENCES

1. Puente, X.S., Sánchez, L.M., Overall, C.M. and López-Otín, C. 2003. Human and mouse proteases: a comparative genomic approach. *Nat. Rev. Genet.* 4: 544-558.
2. Quesada, V., Díaz-Perales, A., Gutierrez-Fernández, A., Garabaya, C., Cal, S. and López-Otín, C. 2004. Cloning and enzymatic analysis of 22 novel human ubiquitin-specific proteases. *Biochem. Biophys. Res. Commun.* 314: 54-62.
3. Hicke, L., Schubert, H.L. and Hill, C.P. 2005. Ubiquitin-binding domains. *Nat. Rev. Mol. Cell Biol.* 6: 610-621.
4. Stegmeier, F., Rape, M., Draviam, V.M., Nalepa, G., Sowa, M.E., Ang, X.L., McDonald, E.R., Li, M.Z., Hannon, G.J., Sorger, P.K., Kirschner, M.W., Harper, J.W. and Elledge, S.J. 2007. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* 446: 876-881.
5. Allen, M.D. and Bycroft, M. 2007. The solution structure of the ZnF UBP domain of USP33/VDU1. *Protein Sci.* 16: 2072-2075.
6. Ha, B.H. and Kim, E.E. 2008. Structures of proteases for ubiquitin and ubiquitin-like modifiers. *BMB Rep.* 41: 435-443.

CHROMOSOMAL LOCATION

Genetic locus: USP45 (human) mapping to 6q16.2.

PRODUCT

USP45 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see USP45 shRNA Plasmid (h): sc-76859-SH and USP45 shRNA (h) Lentiviral Particles: sc-76859-V as alternate gene silencing products.

For independent verification of USP45 (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-76859A, sc-76859B and sc-76859C.

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 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

USP45 siRNA (h) is recommended for the inhibition of USP45 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 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

USP45 (MA44): sc-130478 is recommended as a control antibody for monitoring of USP45 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 USP45 gene expression knockdown using RT-PCR Primer: USP45 (h)-PR: sc-76859-PR (20 μ l). 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.