SANTA CRUZ BIOTECHNOLOGY, INC.

Eap45 siRNA (m): sc-77216

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The Power to Question

BACKGROUND

Vacuolar sorting proteins (VPSs) are required for trafficking normal endocytic and biosynthetic proteins to the vacuole and also play an important role in the budding process of cells. The ESCRT-II (endosomal sorting complex required for transport II) complex, which is involved in endocytosis of ubiquinated membrane proteins, is formed by Eap45, EAP30, and VPS25. These vacuolar sorting proteins are also involved in a multiprotein complex with RNA ploymerase II elongation factor (ELL). Eap45 (ELL-associated protein of 45 kDa), also known as vacuolar protein-sorting-associated protein 36, is a 386 amino acid protein containing a GLUE (GRAM-like ubiquitin-binding in Eap45) domain that mediates the binding to ubiquitin and phophoinosides. Since Eap45 colocalizes with ubiquinated proteins on late endosomes, it is likely that Eap45 plays a role in the endosomal sorting of ubiquniated cargo. There are two isoforms of Eap45 that exist as a result of alternative splicing events.

REFERENCES

1. Martin-Serrano, J., et al. 2003. Divergent retroviral late-budding domains recruit vacuolar protein sorting factors by using alternative adaptor proteins. Proc. Natl. Acad. Sci. USA 100: 12414-12419.
2. Slagsvold, T., et al. 2005. Eap45 in mammalian ESCRT-II binds ubiquitin via a phosphoinositide-interacting GLUE domain. J. Biol. Chem. 280: 19600-19606.
3. Teo, H., et al. 2006. ESCRT-I core and ESCRT-II GLUE domain structures reveal role for GLUE in linking to ESCRT-I and membranes. Cell 125: 99-111.
4. Langelier, C., et al. 2006. Human ESCRT-II complex and its role in human immunodeficiency virus type 1 release. J. Virol. 80: 9465-9480.
5. Alam, S.L., et al. 2006. Structural basis for ubiquitin recognition by the human ESCRT-II EAP45 GLUE domain. Nat. Struct. Mol. Biol. 13: 1029-1030.
6. Hirano, S., et al. 2006. Structural basis of ubiquitin recognition by mammalian Eap45 GLUE domain. Nat. Struct. Mol. Biol. 13: 1031-1032.

CHROMOSOMAL LOCATION

Genetic locus: Vps36 (mouse) mapping to 8 A2.

PRODUCT

Eap45 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 Eap45 shRNA Plasmid (m): sc-77216-SH and Eap45 shRNA (m) Lentiviral Particles: sc-77216-V as alternate gene silencing products.

For independent verification of Eap45 (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-77216A, sc-77216B and sc-77216C.

PROTOCOLS

See our web site at 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 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

Eap45 siRNA (m) is recommended for the inhibition of Eap45 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Eap45 gene expression knockdown using RT-PCR Primer: Eap45 (m)-PR: sc-77216-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.