SANTA CRUZ BIOTECHNOLOGY, INC.

EAP30 siRNA (m): sc-77280

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The Power to Question

BACKGROUND

EAP30 (ELL-associated protein of 30 kDa), also known as SNF8, Dot3 or VPS22, is a 258 amino acid protein that localizes to both the nucleus and the cytoplasm and is a member of the SNF8 family of vacuolar sorting proteins. Expressed as two alternatively spliced isoforms, EAP30 is a component of the multi-protein ESCRT-II complex that is involved in the formation of multivesicular bodies (MVBs) and in the sorting of endosomal cargo proteins within MVBs. In addition to its role in the formation and maintenance of MVBs, the ESCRT-II complex plays a role in targeting proteins to the lysosome for degradation and is also thought to repress the activity of RNA polymerase II (Pol II), thereby regulating transcription. As a member of the ESCRT-II complex, EAP30 is involved in MVB pathways and transcriptional regulation events.

REFERENCES

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3. Martin-Serrano, J., Yarovoy, A., Perez-Caballero, D., Bieniasz, P.D. and Yaravoy, A. 2003. Divergent retroviral late-budding domains recruit vacuolar protein sorting factors by using alternative adaptor proteins. Proc. Natl. Acad. Sci. USA 100: 12414-12419.
4. Wernimont, A.K. and Weissenhorn, W. 2004. Crystal structure of subunit VPS25 of the endosomal trafficking complex ESCRT-II. BMC Struct. Biol. 4: 10.
5. Teo, H., Perisic, O., González, B. and Williams, R.L. 2004. ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. Dev. Cell 7: 559-569.
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CHROMOSOMAL LOCATION

Genetic locus: Snf8 (mouse) mapping to 11 D.

PRODUCT

EAP30 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 EAP30 shRNA Plasmid (m): sc-77280-SH and EAP30 shRNA (m) Lentiviral Particles: sc-77280-V as alternate gene silencing products.

For independent verification of EAP30 (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-77280A, sc-77280B and sc-77280C.

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

EAP30 siRNA (m) is recommended for the inhibition of EAP30 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

EAP30 (C-11): sc-390747 is recommended as a control antibody for monitoring of EAP30 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 EAP30 gene expression knockdown using RT-PCR Primer: EAP30 (m)-PR: sc-77280-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.