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BIG1 siRNA (h): sc-43632

BACKGROUND

Guanine nucleotide-exchange proteins (GEPs) accelerate replacement of bound GDP with GTP and thereby activate ADP-ribosylation factors (ARFs), a family of guanine nucleotide-binding proteins that play an important role in intracellular vesicular trafficking. GEPs comprise two major families, large GEPs that are inhibited by brefeldin A (BFA), a protein that effects Golgi structure and a group of smaller GEPs that are insensitive to BFA. Two genes for GEPs found on human chromosomes 8 and 20 encode BFA sensitive GEPs designated BIG1 and BIG2. Both GEPs contain a sec7 domain that is responsible for their brefeldin inhibition and also their catalytic activity. *In vivo*, BIG1 and BIG2 exist in macromolecular complexes that move between the Golgi membranes and cytosol. BIG2 associates with PKA regulatory subunits, implying that BIG2 may act as an A kinase-anchoring protein (AKAP) that could coordinate the cAMP and ARF regulatory pathways.

REFERENCES

1. Togawa, A., et al. 1999. Purification and cloning of a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP-ribosylation factors. *J. Biol. Chem.* 274: 12308-12315.
2. Li, H., et al. 2003. Protein kinase A-anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). *Proc. Natl. Acad. Sci. USA* 100: 1627-1632.
3. Padilla, P.I., et al. 2003. Interaction of FK506-binding protein 13 with brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1): effects of FK-506. *Proc. Natl. Acad. Sci. USA* 100: 2322-2327.

CHROMOSOMAL LOCATION

Genetic locus: ARFGEF1 (human) mapping to 8q13.2.

PRODUCT

BIG1 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 BIG1 shRNA Plasmid (h): sc-43632-SH and BIG1 shRNA (h) Lentiviral Particles: sc-43632-V as alternate gene silencing products.

For independent verification of BIG1 (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-43632A, sc-43632B and sc-43632C.

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

BIG1 siRNA (h) is recommended for the inhibition of BIG1 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

BIG1 (G-3): sc-376790 is recommended as a control antibody for monitoring of BIG1 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 BIG1 gene expression knockdown using RT-PCR Primer: BIG1 (h)-PR: sc-43632-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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.