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- Expressversand

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# BIG1 shRNA (h) Lentiviral Particles: sc-43632-V

## 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., Morinaga, N., Ogasawara, M., Moss, J. and Vaughan, M. 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., Adamik, R., Pacheco-Rodriguez, G., Moss, J. and Vaughan, M. 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., Chang, M.J., Pacheco-Rodriguez, G., Adamik, R., Moss, J. and Vaughan, M. 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 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see BIG1 siRNA (h): sc-43632 and BIG1 shRNA Plasmid (h): sc-43632-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

BIG1 shRNA (h) Lentiviral Particles is recommended for the inhibition of BIG1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.