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# GAD-25/67 shRNA (h) Lentiviral Particles: sc-43790-V

## BACKGROUND

There are two forms of glutamic acid decarboxylases (GADs) that are found in the brain: GAD-65 (also known as GAD2) and GAD-67 (also known as GAD1, GAD or SCP). GAD-65 and GAD-67 are members of the group II decarboxylase family of proteins and are responsible for catalyzing the rate limiting step in the production of GABA ( $\gamma$ -aminobutyric acid) from L-glutamic acid. Although both GADs are found in the brain, GAD-65 localizes to synaptic vesicle membranes in nerve terminals, while GAD-67 is distributed throughout the cell. GAD-67 is responsible for the basal levels of GABA synthesis. In the case of a heightened demand for GABA in neurotransmission, GAD-65 will transiently activate to assist in GABA production. The loss of GAD-65 is detrimental and can impair GABA neurotransmission, however the loss of GAD-67 is lethal. Due to alternative splicing, two isoforms exist for GAD-67, the predominant GAD-67 form and the minor GAD-25 form. GAD-25 is not expressed in brain but can be found in a variety of endocrine tissues.

## REFERENCES

1. Chessler, S.D., et al. 2002. Immune reactivity to GAD25 in type 1 diabetes mellitus. *Autoimmunity* 35: 335-341.
2. Kanter, I.C., et al. 2007. Cyclophosphamide for anti-GAD antibody-positive refractory status epilepticus. *Epilepsia* 49: 914-920.
3. Korpershoek, E., et al. 2007. Expression of GAD67 and novel GAD67 splice variants during human fetal pancreas development: GAD67 expression in the fetal pancreas. *Endocr. Pathol.* 18: 31-36.
4. Kanaani, J., et al. 2008. A palmitoylation cycle dynamically regulates partitioning of the GABA-synthesizing enzyme GAD65 between ER-Golgi and post-Golgi membranes. *J. Cell Sci.* 121: 437-449.
5. Wei, J. and Wu, J.Y. 2008. Post-translational regulation of L-glutamic acid decarboxylase in the brain. *Neurochem. Res.* 33: 1459-1465.
6. Hwang, I.K., et al. 2008. Comparison of glutamic acid decarboxylase 67 immunoreactive neurons in the hippocampal CA1 region at various age stages in dogs. *Neurosci. Lett.* 431: 251-255.

## CHROMOSOMAL LOCATION

Genetic locus: GAD1 (human) mapping to 2q31.1.

## PRODUCT

GAD-25/67 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 GAD-25/67 siRNA (h): sc-43790 and GAD-25/67 shRNA Plasmid (h): sc-43790-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

GAD-25/67 shRNA (h) Lentiviral Particles is recommended for the inhibition of GAD-25/67 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

GAD-25/67 (H-101): sc-5602 is recommended as a control antibody for monitoring of GAD-25/67 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GAD-25/67 gene expression knockdown using RT-PCR Primer: GAD-25/67 (h)-PR: sc-43790-PR (20  $\mu$ l, 579 bp). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  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.

## PROTOCOLS

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