

GAD-65 siRNA (r): sc-61888

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 (γ -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.

CHROMOSOMAL LOCATION

Genetic locus: *Gad2* (rat) mapping to 17q12.3.

PRODUCT

GAD-65 siRNA (r) 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 GAD-65 shRNA Plasmid (r): sc-61888-SH and GAD-65 shRNA (r) Lentiviral Particles: sc-61888-V as alternate gene silencing products.

For independent verification of GAD-65 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61888A, sc-61888B and sc-61888C.

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

GAD-65 siRNA (r) is recommended for the inhibition of GAD-65 expression in rat 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

GAD-65 (A-3): sc-377145 is recommended as a control antibody for monitoring of GAD-65 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 GAD-65 gene expression knockdown using RT-PCR Primer: GAD-65 (r)-PR: sc-61888-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.