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GCN2 siRNA (m): sc-45645

BACKGROUND

The family of stress-responsive protein kinases include HRI (heme-regulated inhibitor or EIF2AK1), PKR (EIF2AK2 or TIK), PERK (EIF2AK3) and GCN2 (EIF2AK4). These proteins phosphorylate the eukaryotic translation initiation factor 2 α (eIF2 α) on Ser 51 to regulate general and gene-specific protein synthesis. Phosphorylated eIF2 α acts as an inhibitor of its guanine nucleotide exchange factor eIF2B. GCN2, a unique eIF2 α kinase, exists in all eukaryotes from yeast to mammals. In mammals, expression of GCN2 is highest in liver and brain tissues. GCN2 primarily initiates the phosphorylation of eIF2 α in response to UV, but has been shown to increase phosphorylation activity in response to serum starvation. Also, substitution of Asp 83 for Ala on eIF2 α results in impaired phosphorylation by GCN2 and PKR, suggesting a contribution of remote residues to kinase-substrate recognition.

REFERENCES

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- Jiang, H.Y., et al. 2003. Phosphorylation of the α subunit of eukaryotic initiation factor 2 is required for activation of NF κ B in response to diverse cellular stresses. *Mol. Cell. Biol.* 23: 5651-5663.
- Anthony, T.G., et al. 2004. Preservation of liver protein synthesis during dietary leucine deprivation occurs at the expense of skeletal muscle mass in mice deleted for eIF2 kinase GCN2. *J. Biol. Chem.* 279: 36553-36561.
- Costa-Mattioli, M., et al. 2005. Translational control of hippocampal synaptic plasticity and memory by the eIF2 α kinase GCN2. *Nature* 436: 1166-1173.
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- Hamanaka, R.B., et al. 2005. PERK and GCN2 contribute to eIF2 α phosphorylation and cell cycle arrest after activation of the unfolded protein response pathway. *Mol. Biol. Cell* 16: 5493-5501.
- Jiang, H.Y., et al. 2005. GCN2 phosphorylation of eIF2 α activates NF κ B in response to UV irradiation. *Biochem. J.* 385: 371-380.

CHROMOSOMAL LOCATION

Genetic locus: Eif2ak4 (mouse) mapping to 2 E5.

PRODUCT

GCN2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCN2 shRNA Plasmid (m): sc-45645-SH and GCN2 shRNA (m) Lentiviral Particles: sc-45645-V as alternate gene silencing products.

For independent verification of GCN2 (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-45645A, sc-45645B and sc-45645C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

GCN2 siRNA (m) is recommended for the inhibition of GCN2 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GCN2 gene expression knockdown using RT-PCR Primer: GCN2 (m)-PR: sc-45645-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.

SELECT PRODUCT CITATIONS

- Okada, A., et al. 2017. D-serine, a novel uremic toxin, induces senescence in human renal tubular cells via GCN2 activation. *Sci. Rep.* 7: 11168.
- Shi, W.Z., et al. 2019. GCN2 suppression attenuates cerebral ischemia in mice by reducing apoptosis and endoplasmic reticulum (ER) stress through the blockage of FoxO3a-regulated Ros production. *Biochem. Biophys. Res. Commun.* 516: 285-292.

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.