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GCN2 shRNA (h) Lentiviral Particles: sc-45644-V

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

- Berlanga, J.J., et al. 1999. Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2 α kinase. *Eur. J. Biochem.* 265: 754-762.
- 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-Mattoli, M., et al. 2005. Translational control of hippocampal synaptic plasticity and memory by the eIF2 α kinase GCN2. *Nature* 436: 1166-1173.

CHROMOSOMAL LOCATION

Genetic locus: EIF2AK4 (human) mapping to 15q15.1.

PRODUCT

GCN2 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 μ l frozen stock containing 1.0×10^6 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see GCN2 siRNA (h): sc-45644 and GCN2 shRNA Plasmid (h): sc-45644-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

GCN2 shRNA (h) Lentiviral Particles is recommended for the inhibition of GCN2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

GCN2 (F-7): sc-374609 is recommended as a control antibody for monitoring of GCN2 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 GCN2 gene expression knockdown using RT-PCR Primer: GCN2 (h)-PR: sc-45644-PR (20 μ l, 461 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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