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SANTA CRUZ BIOTECHNOLOGY, INC.

GCN2 siRNA (h): sc-45644

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 2α (eIF2α) on Ser 51 to regulate general and gene-specific protein synthesis. Phosphorylated eIF2α acts as an inhibitor of its guanine nucleotide exchange factor eIF2B. GCN2, a unique eIF2α 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 eIF2α 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 eIF2α results in impaired phosphorylation by GCN2 and PKR, suggesting a contribution of remote residues to kinase-substrate recognition.

CHROMOSOMAL LOCATION

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

PRODUCT

GCN2 siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCN2 shRNA Plasmid (h): sc-45644-SH and GCN2 shRNA (h) Lentiviral Particles: sc-45644-V as alternate gene silencing products.

For independent verification of GCN2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45644A, sc-45644B and sc-45644C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GCN2 siRNA (h) is recommended for the inhibition of GCN2 expression in human 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 µM in 66 µl. 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

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 GCN2 gene expression knockdown using RT-PCR Primer: GCN2 (h)-PR: sc-45644-PR (20 µl, 461 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lu, W., et al. 2009. The role of nitric-oxide synthase in the regulation of UVB light-induced phosphorylation of the α subunit of eukaryotic initiation factor 2. J. Biol. Chem. 284: 24281-24288.
2. García-Navas, R., et al. 2012. Depletion of L-arginine induces autophagy as a cytoprotective response to endoplasmic reticulum stress in human T lymphocytes. Autophagy 8: 1557-1576.
3. Wei, C., et al. 2015. Involvement of general control nonderepressible kinase 2 in cancer cell apoptosis by posttranslational mechanisms. Mol. Biol. Cell 26: 1044-1057.
4. Chaveroux, C., et al. 2016. Nutrient shortage triggers the hexosamine biosynthetic pathway via the GCN2-ATF4 signalling pathway. Sci. Rep. 6: 27278.
5. Fu, X., et al. 2016. Malonate induces the assembly of cytoplasmic stress granules. FEBS Lett. 590: 22-33.
6. Bretin, A., et al. 2016. Activation of the EIF2AK4-EIF2A/eIF2α-ATF4 pathway triggers autophagy response to Crohn disease-associated adherent-invasive *Escherichia coli* infection. Autophagy 12: 770-783.
7. Cheng, C.T., et al. 2018. Arginine starvation kills tumor cells through aspartate exhaustion and mitochondrial dysfunction. Commun. Biol. 1: 178.
8. Timalsina, S., et al. 2018. Chemical compounds that suppress hypoxia-induced stress granule formation enhance cancer drug sensitivity of human cervical cancer HeLa cells. J. Biochem. 164: 381-391.
9. Ge, L., et al. 2018. GCN2 is a potential prognostic biomarker for human papillary renal cell carcinoma. Cancer Biomark. 22: 395-403.

RESEARCH USE

For research use only, not for use in diagnostic procedures.