

Gas2 siRNA (m): sc-62369

BACKGROUND

Gas2 is a 313 amino acid protein encoded by the human gene GAS2. Gas2 is thought to play a role in apoptosis by acting as a cell death substrate for caspases. Gas2, a component of the microfilament system, is cleaved by a caspase (caspase-3 and caspase-7) at Asparagine 278 during apoptosis. The cleaved form resulting from this dramatically induces the rearrangement of the Actin cytoskeleton and causes potent changes in the shape of the affected cells. Gas2 is believed to also be involved in the membrane ruffling process. During the G₀-G₁ transition phase Gas2 can be found phosphorylated on its serine residues. Gas2 is a cytoskeleton and peripheral membrane protein that co-localizes with Actin fibers at the cell border and along the stress fibers in growth-arrested fibroblasts. Gas2 is mainly membrane-associated but when hyperphosphorylated it will accumulate at membrane ruffles. Gas2 is specifically expressed at growth arrest and is ubiquitously expressed with highest levels found in liver, lung and kidney. There is no evidence, however, of Gas2 expression in spleen.

REFERENCES

1. Fleming, J.V., et al. 1998. Effects of nutrient deprivation and differentiation on the expression of growth-arrest genes (gas and gadd) in F9 embryonal carcinoma cells. *Biochem. J.* 330: 573-579
2. Collavin, L., et al. 1998. cDNA characterization and chromosome mapping of the human GAS2 gene. *Genomics* 48: 265-269.
3. Sgorbissa, A., et al. 2000. Caspase-3 and caspase-7 but not caspase-6 cleave Gas2 in vitro: implications for microfilament reorganization during apoptosis. *J. Cell Sci.* 112: 4475-4482
4. Benetti, R., et al. 2001. The death substrate Gas2 binds m-calpain and increases susceptibility to p53-dependent apoptosis. *EMBO J.* 20: 2702-2714.
5. Goriounov, D., et al. 2003. Protein products of human Gas2-related genes on chromosomes 17 and 22 (hGAR17 and hGAR22) associate with both microfilaments and microtubules. *J. Cell Sci.* 116: 1045-1058.
6. Brockman, J.L., et al. 2005. Prolactin signals via Stat5 and Oct-1 to the proximal cyclin D1 promoter. *Mol. Cell. Endocrinol.* 239: 45-53.

CHROMOSOMAL LOCATION

Genetic locus: Gas2 (mouse) mapping to 7 B5.

PRODUCT

Gas2 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 Gas2 shRNA Plasmid (m): sc-62369-SH and Gas2 shRNA (m) Lentiviral Particles: sc-62369-V as alternate gene silencing products.

For independent verification of Gas2 (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-62369A, sc-62369B and sc-62369C.

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

Gas2 siRNA (m) is recommended for the inhibition of Gas2 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.

GENE EXPRESSION MONITORING

Gas2 (Y-19): sc-54809 is recommended as a control antibody for monitoring of Gas2 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Gas2 gene expression knockdown using RT-PCR Primer: Gas2 (m)-PR: sc-62369-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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