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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

G2A siRNA (h): sc-43776

BACKGROUND

G2A (for G₂ accumulation) is a seven transmembrane G protein-coupled receptor that is upregulated in response to DNA damage and stress. G2A is predominantly expressed in hematopoietic tissues and in hematopoietic stem cells, and it is more highly detected in pro-B cells, while lower expression is observed in immature B cells and pre-B cells. G2A is expressed throughout T cell maturation, and it is further increased in response to T-cell activation. Ectopic expression of a G2A fusion protein in NIH/3T3 fibroblasts induces a cell cycle arrest that is consistent with a block at the G₂/M transition. G2A is also able to attenuate the proliferative effects of Bcr-Abl, a chimeric tyrosine kinase oncogene, suggesting that G2A possesses anti-oncogenic properties. The amino acid sequence of G2A contains a destruction box motif that is consistently observed in cyclins, where it is required for ubiquitination and proteolytic degradation.

REFERENCES

1. Weng, Z., et al. 1998. A DNA damage and stress inducible G protein-coupled receptor blocks cells in G₂/M. *Proc. Natl. Acad. Sci. USA* 95: 12334-12339.
2. Zohn, I.E., et al. 2000. G2A is an oncogenic G protein-coupled receptor. *Oncogene* 19: 3866-3877.
3. Kabarowski, J.H., et al. 2001. Lysophosphatidylcholine as a ligand for the immunoregulatory receptor G2A. *Science* 293: 702-705.
4. Rikitake, Y., et al. 2002. Expression of G2A, a receptor for lysophosphatidylcholine, by macrophages in murine, rabbit, and human atherosclerotic plaques. *Arterioscler. Thromb. Vasc. Biol.* 22: 2049-2053.
5. Lin, P. and Ye, R.D. 2003. The lysophospholipid receptor G2A activates a specific combination of G proteins and promotes apoptosis. *J. Biol. Chem.* 278: 14379-14386.

CHROMOSOMAL LOCATION

Genetic locus: GPR132 (human) mapping to 14q32.33.

PRODUCT

G2A 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see G2A shRNA Plasmid (h): sc-43776-SH and G2A shRNA (h) Lentiviral Particles: sc-43776-V as alternate gene silencing products.

For independent verification of G2A (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-43776A, sc-43776B and sc-43776C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

G2A siRNA (h) is recommended for the inhibition of G2A 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 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

G2A (G-5): sc-137112 is recommended as a control antibody for monitoring of G2A 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 G2A gene expression knockdown using RT-PCR Primer: G2A (h)-PR: sc-43776-PR (20 μ l, 533 bp). 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.