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SANTA CRUZ BIOTECHNOLOGY, INC.

G2A siRNA (m): sc-44371

BACKGROUND

G2A (for G_2 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 G_2/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. Bedi, A., et al. 1995. Bcr-Abl-mediated inhibition of apoptosis with delay of G_2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood 86: 1148-1158.
2. Allday, M.J., et al. 1995. DNA damage in human B cells can induce apoptosis, proceeding from G_1/S when p53 is transactivation competent and G_2/M when it is transactivation defective. EMBO J. 14: 4994-5005.
3. Hochstrasser, M. 1996. Ubiquitin-dependent protein degradation. Annu. Rev. Genet. 30: 405-439.
4. Weng, Z., et al. 1998. A DNA damage and stress inducible G protein-coupled receptor blocks cells in G_2/M. Proc. Natl. Acad. Sci. USA 95: 12334-12339.
5. Shimizu, A., et al. 1998. Cyclin G contributes to G_2/M arrest of cells in response to DNA damage. Biochem. Biophys. Res. Commun. 242: 529-533.
6. Aguda, B.D. 1999. A quantitative analysis of the kinetics of the G_2 DNA damage checkpoint system. Proc. Natl. Acad. Sci. USA 96: 11352-11357.

CHROMOSOMAL LOCATION

Genetic locus: Gpr132 (mouse) mapping to 12 F1.

PRODUCT

G2A 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see G2A shRNA Plasmid (m): sc-44371-SH and G2A shRNA (m) Lentiviral Particles: sc-44371-V as alternate gene silencing products.

For independent verification of G2A (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-44371A, sc-44371B and sc-44371C.

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 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

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

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-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 G2A gene expression knockdown using RT-PCR Primer: G2A (m)-PR: sc-44371-PR (20 µl, 588 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.