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G2A shRNA (m) Lentiviral Particles: sc-44371-V

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. Bedi, A., et al. 1995. Bcr-Abl-mediated inhibition of apoptosis with delay of G₂/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 G₁/S when p53 is transactivation competent and G₂/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 G₂/M. *Proc. Natl. Acad. Sci. USA* 95: 12334-12339.

CHROMOSOMAL LOCATION

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

PRODUCT

G2A shRNA (m) 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 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see G2A siRNA (m): sc-44371 and G2A shRNA Plasmid (m): sc-44371-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.

PROTOCOLS

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

APPLICATIONS

G2A shRNA (m) Lentiviral Particles is recommended for the inhibition of G2A expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

G2A (A-20): sc-23810 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 (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 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.

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.