SANTA CRUZ BIOTECHNOLOGY, INC.

GADD 45α siRNA (m): sc-35439

BACKGROUND

It is well established that cell cycle progression is subject to arrest at G_1 and G_2 checkpoints in response to DNA damage, presumably to allow time for DNA repair prior to entry into S and M phase, respectively. The p53 tumor suppressor is required for one such G_1 checkpoint and functions to upregulate expression of GADD 45 and p21. p21 functions to inhibit the kinase activity of multiple Cdk complexes, which may account for its suppression of cell growth. GADD 45 binds both Cdks and PCNA, a protein involved in DNA replication and repair. GADD 45 has been shown to stimulate DNA excision repair *in vitro* and to inhibit entry of cells into S phase. Thus, it has been suggested that GADD 45 may serve as a link between p53-dependent cell cycle checkpoint and DNA repair.

REFERENCES

1. Murray, A.W. 1992. Creative blocks: cell-cycle checkpoints and feedback controls. Nature 359: 599-604.
2. Kuerbitz, S.J., et al. 1992. Wildtype p53 is a cell cycle checkpoint determinant following irradiation. Proc. Natl. Acad. Sci. USA 89: 7491-7495.
3. Kastan, M.B., et al. 1992. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD 45 is defective in ataxia-telangiectasia. Cell 71: 587-597.
4. Harper, J.W., et al. 1993. The p21 Cdk-interacting protein CIP1 is a potent inhibitor of G_1 cyclin-dependent kinases. Cell 75: 805-816.
5. El-Deiry, W.S., et al. 1994. WAF1/CIP1 is induced in p53-mediated G_1 arrest and apoptosis. Cancer Res. 54: 1169-1174.

CHROMOSOMAL LOCATION

Genetic locus: Gadd45a (mouse) mapping to 6 C1.

PRODUCT

GADD 45α 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 GADD 45α shRNA Plasmid (m): sc-35439-SH and GADD 45α shRNA (m) Lentiviral Particles: sc-35439-V as alternate gene silencing products.

For independent verification of GADD 45α (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-35439A, sc-35439B and sc-35439C.

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

GADD 45α siRNA (m) is recommended for the inhibition of GADD 45α 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

GADD 45α (C-4): sc-6850 is recommended as a control antibody for monitoring of GADD 45α 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 GADD 45α gene expression knockdown using RT-PCR Primer: GADD 45α (m)-PR: sc-35439-PR (20 µl, 400 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Choi, H.J., et al. 2011. Critical role of the JNK-p53-GADD45α apoptotic cascade in mediating oxidative cytotoxicity in hippocampal neurons. Br. J. Pharmacol. 162: 175-192.
2. Choi, H.J., et al. 2020. 4-hydroxyestrone, an endogenous estrogen metabolite, can strongly protect neuronal cells against oxidative damage. Sci. Rep. 10: 7283.

RESEARCH USE

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

PROTOCOLS

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