

GADD 45 γ siRNA (r): sc-63316

BACKGROUND

Cell cycle progression is subject to arrest at G₁ and G₂ 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 G₁ checkpoint and functions to upregulate expression of GADD 45 and p21. GADD 45 binds both Cdks and PCNA, a protein involved in DNA replication and repair. GADD 45 stimulates DNA excision repair *in vitro* and inhibits entry of cells into S phase. Thus, it has been suggested that GADD 45 may serve as a link between the p53-dependent cell cycle checkpoint and DNA repair. GADD 45-like proteins, GADD 45 β and GADD 45 γ , have been shown to be induced by environmental stresses. GADD 45 β and GADD 45 γ are thought to induce p38/JNK activation via MEKK 4 activation.

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. Wild-type 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. Smith, M.L., et al. 1994. Interaction of the p53-regulated protein GADD 45 with proliferating cell nuclear antigen. *Science* 266: 1376-1379.
5. Marx, J. 1994. New link found between p53 and DNA repair. *Science* 266: 1321-1322.
6. Takekawa, M., et al. 1998. A family of stress-inducible GADD 45-like proteins mediate activation of the stress-responsive MTK1/MEKK 4 MAPKKK. *Cell* 95: 521-530.
7. Zerbini, L.F., et al. 2004. NF κ B-mediated repression of growth arrest- and DNA-damage-inducible proteins 45 α and γ is essential for cancer cell survival. *Proc. Natl. Acad. Sci. USA* 101: 13618-13623.

CHROMOSOMAL LOCATION

Genetic locus: Gadd45g (rat) mapping to 17p14.

PRODUCT

GADD 45 γ siRNA (r) 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 GADD 45 γ shRNA Plasmid (r): sc-63316-SH and GADD 45 γ shRNA (r) Lentiviral Particles: sc-63316-V as alternate gene silencing products.

For independent verification of GADD 45 γ (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63316A, sc-63316B and sc-63316C.

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

GADD 45 γ siRNA (r) is recommended for the inhibition of GADD 45 γ expression in rat 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

GADD 45 γ (B-1): sc-393261 is recommended as a control antibody for monitoring of GADD 45 γ 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 GADD 45 γ gene expression knockdown using RT-PCR Primer: GADD 45 γ (r)-PR: sc-63316-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.