

GADD 45 β siRNA (m): sc-37417

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 MEKK4 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. Marx, J. 1994. New link found between p53 and DNA repair. *Science* 266: 1321-1322.
5. Smith, M.L., et al. 1994. Interaction of the p53-regulated protein GADD 45 with proliferating cell nuclear antigen. *Science* 266: 1376-1379.
6. Takekawa, M., et al. 1998. A family of stress-inducible GADD 45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. *Cell* 95: 521-530.

CHROMOSOMAL LOCATION

Genetic locus: Gadd45b (mouse) mapping to 10 C1.

PRODUCT

GADD 45 β 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GADD 45 β shRNA Plasmid (m): sc-37417-SH and GADD 45 β shRNA (m) Lentiviral Particles: sc-37417-V as alternate gene silencing products.

For independent verification of GADD 45 β (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-37417A, sc-37417B and sc-37417C.

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.

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 β siRNA (m) is recommended for the inhibition of GADD 45 β 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 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 β (G-11): sc-377311 is recommended as a control antibody for monitoring of GADD 45 β 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 β gene expression knockdown using RT-PCR Primer: GADD 45 β (m)-PR: sc-37417-PR (20 μ l, 527 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.