

# GADD 45 $\beta$ siRNA (r): sc-270211

## BACKGROUND

Cell cycle progression is subject to arrest at G<sub>1</sub> and G<sub>2</sub> 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<sub>1</sub> 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 $\beta$  and GADD 45 $\gamma$ , have been shown to be induced by environmental stresses. GADD 45 $\beta$  and GADD 45 $\gamma$  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.

## CHROMOSOMAL LOCATION

Genetic locus: Gadd45b (rat) mapping to 7q11.

## PRODUCT

GADD 45 $\beta$  siRNA (r) is a pool of 2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GADD 45 $\beta$  shRNA Plasmid (r): sc-270211-SH and GADD 45 $\beta$  shRNA (r) Lentiviral Particles: sc-270211-V as alternate gene silencing products.

For independent verification of GADD 45 $\beta$  (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-270211A and sc-270211B.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

GADD 45 $\beta$  siRNA (r) is recommended for the inhibition of GADD 45 $\beta$  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  $\mu$ M in 66  $\mu$ 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 $\beta$  (G-11): sc-377311 is recommended as a control antibody for monitoring of GADD 45 $\beta$  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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 $\beta$  gene expression knockdown using RT-PCR Primer: GADD 45 $\beta$  (r)-PR: sc-270211-PR (20  $\mu$ l, 484 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Kigar, S.L., et al. 2015. Gadd45b is an epigenetic regulator of juvenile social behavior and alters local pro-inflammatory cytokine production in the rodent amygdala. *Brain Behav. Immun.* 46: 60-69.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.