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Claspin siRNA (h): sc-45412

BACKGROUND

Claspin, an essential replication checkpoint control protein, regulates the interaction between Chk1 and the upstream regulatory kinase ATR. Chk1 mediates cell cycle arrest in response to a block in DNA replication or to DNA damage by ultraviolet radiation. Claspin becomes phosphorylated within its Chk1 binding domain in response to replication stress. This domain consists of two highly conserved repeats of approximately ten amino acids. Each repeat contains a serine residue (Serine 864 and Serine 895) that undergoes phosphorylation. Binding of Chk1 and Claspin promotes the interaction between Chk1 and ATR and Rad9, thereby arresting the cell cycle. Claspin is most abundant within cells at the S/G₂ phase.

REFERENCES

1. Kumagai, A., et al. 2000. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in *Xenopus* egg extracts. *Mol. Cell* 6: 839-849.
2. Chini, C.C., et al. 2003. Human Claspin is required for replication checkpoint control. *J. Biol. Chem.* 278: 30057-30062.
3. Jeong, S.Y., et al. 2003. Phosphorylated Claspin interacts with a phosphate-binding site in the kinase domain of Chk1 during ATR-mediated activation. *J. Biol. Chem.* 278: 46782-46788.
4. Kumagai, A., et al. 2003. Repeated phosphopeptide motifs in Claspin mediate the regulated binding of Chk1. *Nat. Cell Biol.* 5: 161-165.
5. Sar, F., et al. 2004. Human Claspin is a ring-shaped DNA-binding protein with high affinity to branched DNA structures. *J. Biol. Chem.* 279: 39289-39295.
6. Lin, S.Y., et al. 2004. Human Claspin works with BRCA1 to both positively and negatively regulate cell proliferation. *Proc. Natl. Acad. Sci. USA* 101: 6484-6489.

CHROMOSOMAL LOCATION

Genetic locus: CLSPN (human) mapping to 1p34.3.

PRODUCT

Claspin siRNA (h) 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 Claspin shRNA Plasmid (h): sc-45412-SH and Claspin shRNA (h) Lentiviral Particles: sc-45412-V as alternate gene silencing products.

For independent verification of Claspin (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45412A, sc-45412B and sc-45412C.

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

Claspin siRNA (h) is recommended for the inhibition of Claspin expression in human 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

Claspin (B-6): sc-376773 is recommended as a control antibody for monitoring of Claspin 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 Claspin gene expression knockdown using RT-PCR Primer: Claspin (h)-PR: sc-45412-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.