

p-CstF-64 (Ser 83)-R: sc-16480-R

BACKGROUND

Polyadenylation of mRNA precursors is a two-step reaction that requires multiple protein factors. The first step, endo-nucleolytic cleavage of polyadenylation substrates, requires CstF (cleavage stimulation factor), a heterotrimer. CstF-64 contains an RNA binding domain, which is responsible for the RNA binding activity of CstF. CstF-64 is expressed in all somatic cells and in pre and postmeiotic, but not meiotic, germ cells. CstF-64 displays size heterogeneity on SDS-PAGE, due at least in part to phosphorylation. One of these phosphorylation sites, Ser 83, resides in a possible PKC consensus site of the RNA binding domain. The other two phosphorylation sites, Ser 364 and Ser 498, reside within the possible cGMP-dependent protein kinase sites.

REFERENCES

1. Takagaki, Y., Manley, J.L., MacDonald, C.C., Wilusz, J. and Shenk, T. 1990. A multisubunit factor, CstF, is required for polyadenylation of mammalian pre-mRNAs. *Genes Dev.* 4: 2112-2120.
2. Takagaki, Y., MacDonald, C.C., Shenk, T. and Manley, J.L. 1992. The human 64 kDa polyadenylation factor contains a ribonucleoprotein-type RNA binding domain and unusual auxiliary motifs. *Proc. Natl. Acad. Sci. USA* 89: 1403-1407.
3. Kleiman, F.E. and Manley, J.L. 1999. Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. *Science* 285: 1576-1579.
4. Takagaki, Y. and Manley, J.L. 2000. Complex protein interactions within the human polyadenylation machinery identify a novel component. *Mol. Cell Biol.* 20: 1515-1525.
5. Hatton, L.S., Eloranta, J.J., Figueiredo, L.M., Takagaki, Y., Manley, J.L. and O'Hare, K. 2000. The *Drosophila* homologue of the 64 kDa subunit of cleavage stimulation factor interacts with the 77 kDa subunit encoded by the suppressor of forked gene. *Nucleic Acids Res.* 28: 520-526.

CHROMOSOMAL LOCATION

Genetic locus: CSTF2 (human) mapping to Xq22.1; Cstf2 (mouse) mapping to X E3.

SOURCE

p-CstF-64 (Ser 83)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 83 of CstF-64 of human origin.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-16480 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

p-CstF-64 (Ser 83)-R is recommended for detection of Ser 83 phosphorylated CstF-64 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-CstF-64 (Ser 83)-R is also recommended for detection of correspondingly phosphorylated Ser on CstF-64 in additional species, including equine, canine, bovine, porcine and avian.

Suitable for use as control antibody for CstF-64 siRNA (h): sc-35118, CstF-64 siRNA (m): sc-35119, CstF-64 shRNA Plasmid (h): sc-35118-SH, CstF-64 shRNA Plasmid (m): sc-35119-SH, CstF-64 shRNA (h) Lentiviral Particles: sc-35118-V and CstF-64 shRNA (m) Lentiviral Particles: sc-35119-V.

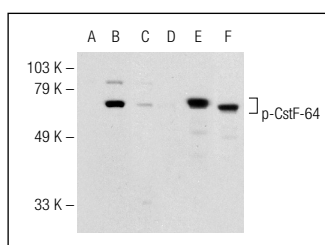
Molecular Weight of p-CstF-64: 64 kDa.

Positive Controls: CstF-64T (h): 293T Lysate: sc-114748 or HeLa whole cell lysate: sc-2200.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent) and Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



Western blot analysis of CstF-64 phosphorylation in non-transfected: sc-117752 (A,D), untreated human CstF-64T transfected: sc-114748 (B,E) and lambda protein phosphatase (sc-200312A) treated human CstF-64T transfected: sc-114748 (C,F) 293T whole cell lysates. Antibodies tested include p-CstF-64 (Ser 83)-R: sc-16480-R (A,B,C) and CstF-64 (H-300): sc-28201 (D,E,F).

RESEARCH USE

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