# p-Chk1 (Ser 280): sc-135636



The Power to Question

#### **BACKGROUND**

Cell cycle events are regulated by the sequential activation and deactivation of cyclin dependent kinases (Cdks) and by proteolysis of cyclins. Chk1 and Chk2 are involved in these processes as regulators of Cdks. Chk1 and Chk2 both function as essential components in the  $\rm G_2$  DNA damage checkpoint by phosphorylating Cdc25C in response to DNA damage. Phosphorylation inhibits Cdc25C activity, thereby blocking mitosis. Cdc25A, Cdc25B and Cdc25C protein tyrosine phosphatases function as mitotic activators by dephosphorylating Cdc2 p34 on regulatory tyrosine residues. It has also been shown that Chk1 can phosphorylate Wee 1 *in vitro*, providing evidence that the hyperphosphorylated form of Wee 1, seen in cells delayed by Chk1 overexpression, is due to phosphorylation by Chk1. Chk1 is phosphorylated on Serine 345 (S345) in response to UV, IR and hydroxyurea (HU). Chk1 plays an essential role in the mammalian DNA damage checkpoint, embryonic development and tumor suppression. The phosphorylation of human Chk1 on Ser 280 is thought to be promoted by Akt1 and may lead to mono and/or diubiquitination of Chk1.

#### **REFERENCES**

- Gautier, J., Solomon, M.J., Booher, R.N., Bazan, J.F. and Kirschner, M.W. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates Cdc2 p34. Cell 67: 197-211.
- 2. Barinaga, M. 1995. A new twist to the cell cycle. Science 269: 631-632.
- Sanchez, Y., Wong, C., Thoma, R.S., Richman, R., Wu, Z., Piwnica-Worms, H. and Elledge, S.J. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 277: 1497-1501.
- O'Connell, M.J., Raleigh, J.M., Verkade, H.M. and Nurse, P. 1997. Chk1 is a Wee 1 kinase in the G<sub>2</sub> DNA damage checkpoint inhibiting Cdc2 by Y15 phosphorylation. EMBO J. 16: 545-554.
- 5. Peng, C.Y., Graves, P.R., Thoma, R.S., Wu, Z., Shaw, A.S. and Piwnica-Worms, H. 1997. Mitotic and  $\rm G_2$  checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 277: 1501-1505.
- Matsuoka, S., Huang, M. and Elledge, S.J. 1998. Linkage of Atm to cell cycle regulation by the Chk2 protein kinase. Science 282: 1893-1897.
- 7. Liu, Q., Guntuku, S., Cui, X.S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, L.A. and Elledge, S.J. 2000. Chk1 is an essential kinase that is regulated by ATR and required for the  $\rm G_2/M$  DNA damage checkpoint. Genes Dev. 14: 1448-1459.

## CHROMOSOMAL LOCATION

Genetic locus: CHEK1 (human) mapping to 11q24.2.

## SOURCE

p-Chk1 (Ser 280) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 280 of Chk1 of human origin.

#### **RESEARCH USE**

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

#### **PRODUCT**

Each vial contains 100  $\mu g$  IgG in 1.0 ml PBS with < 0.1% sodium azide and 0.1% gelatin.

#### **APPLICATIONS**

p-Chk1 (Ser 280) is recommended for detection of Ser 280 phosphorylated Chk1 of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunoprecipitation [1-2 μg per 100-500 μg of total protein (1 ml of cell lysate)].

Suitable for use as control antibody for Chk1 siRNA (h): sc-29269, Chk1 shRNA Plasmid (h): sc-29269-SH and Chk1 shRNA (h) Lentiviral Particles: sc-29269-V.

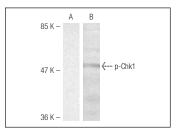
Molecular Weight of p-Chk1: 56 kDa.

Positive Controls: UV treated HL-60 cell extract.

#### **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. 2) 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



p-Chk1 (Ser 280): sc-135636. Western blot analysis of phosphorylated Chk1 expression in untreated (**A**) and UV-treated (**B**) HL-60 cell extracts.

#### **STORAGE**

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

## **PROTOCOLS**

See our web site at www.scbt.com or our catalog for detailed protocols and support products.