Phospho-Chk2 (Thr432) Antibody

- **Store at** -20°C
- **100 µl** (10 Western mini-blots)

**Applications**

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**Background:** Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1–3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) each followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (4,5). After DNA damage by ionizing radiation (IR), UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR (5–7). The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to auto-phosphorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain (8).

**Specificity/Sensitivity:** Phospho-Chk2 (Thr432) Antibody detects endogenous levels of Chk2 only when phosphorylated at threonine 432. The antibody does not cross-react with Chk2 phosphorylated at other sites.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr432 of human Chk2. Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**


**Background References:**


**Recommended Antibody Dilutions:** Western blotting 1:1000

**Companion Products:**

- Phospho-Chk2 (Thr68) Antibody #2661
- Phospho-Chk2 (Ser33/35) Antibody #2665
- Phospho-Chk2 (Ser19) Antibody #2666
- Phospho-Chk2 (Thr387) Antibody #2668

**Anti-rabbit IgG, HRP-linked Antibody #7074**

**Store:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C.

**Do not aliquot the antibody.**

**Recommended Antibody Dilutions:** Western blotting 1:1000

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**Anti-rabbit IgG, HRP-linked Antibody #7074**

**Biotinylated Protein Ladder Detection Pack #7727**

**20X LumiGLO® Reagent and 20X Peroxide #7003**

**Applications Key:**

- **W**—Western
- **IP**—Immunoprecipitation
- **IC**—Immunohistochemistry
- **IC**—Immunocytochemistry
- **IF**—Immunofluorescence
- **F**—Flow cytometry
- **E**—ELISA
- **O**—OELFA

**Species Cross-Reactivity Key:**

- **H**—human
- **M**—mouse
- **R**—rat
- **Hm**—hamster
- **Mk**—monkey
- **Mi**—mink
- **C**—chicken
- **X**—Xenopus
- **Z**—zebra fish
- **B**—bovine
- **All**—all species expected

**IMPORTANT:** For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.
Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. 1X SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. 10X Tris Buffered Saline (TBS): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. Wash Buffer: 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. Phototope®-HRP Western Blot Detection System #7071: Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.