



Phospho-Cdc25C Ser216 Mouse Monoclonal Antibody

Product Data Sheet

For Research Use Only, Not for use in diagnostic procedures

Phospho-Cdc25C Ser216 Mouse Monoclonal Antibody (Clone TK-1F1) Cat# CY-M1018

100 µg (1 mg/ml x 100 µL)

Clone Name	Applications	Species Cross-reactivity	Molecular Wt.	Source Isotype
TK-1F1	WB, E	H	54 kDa	Mouse IgG1

Background: The mitotic inducer Cdc25 phosphatase controls the activation of Cdc2/cyclin-B protein kinase and entry into mitosis in eukaryotic cells. Cdc25C is highly regulated by multiple post-translational modifications within its N-terminal regulatory domain and site-specific protein interactions. Phosphorylation of one inhibitory site, Ser216 targeted by multiple kinases determines the timing of Cdc25C activation and arrests cells in G2 in response to checkpoint, stress, developmental and extracellular signals. In mitosis, phosphorylation of several Ser/Thr residues and Pin1-catalysed peptidyl-proline isomerisation produces activation. Phosphorylation of one activating site is antagonistic to the proximal inhibitory site and maintains Cdc25C activity during mitosis. Phosphorylation and interacting proteins also modulate the nuclear import and export signals on Cdc25C, inducing dramatic changes in its localization within the cell. Thus, the regulation of Cdc25C activity and localization integrates multiple signals that govern the decision to enter mitosis.

Specificity/Sensitivity: Phospho-Cdc25C Ser216 Antibody detects phosphorylated recombinant Cdc25C only when phosphorylated at serine216 *in vitro*, by western blotting.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a synthetic phosphopeptide corresponding to residues surrounding Ser216 of human Cdc25C. IgG is purified by protein A-sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL, ELISA for detection of Chk1, Chk2 and C-TAK1 kinase activity: 1 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB:Western Blotting IP:Immunoprecipitation IHC:Immunohistochemistry IC:Immunocytochemistry F:Flow cytometry E:ELISA FP:Fluorescence Polarization assay

Species Cross-Reactivity Key: H:human M:mouse R:rat Hm:hamster Mk:monkey Mi:mink C:chicken X:*Xenopus* Z:zebra fish All:all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



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Selected Application References:

1. Sadhu, K.; Reed, S. I.; Richardson, H.; Russell, P. Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G(2). *Proc. Nat. Acad. Sci.* **87**: 5139-5143, 1990.
2. Strausfeld, U.; Labbe, J. C.; Fesquet, D.; Cavadore, J. C.; Picard, A.; Sadhu, K.; Russell, P.; Doree, M. Dephosphorylation and activation of a p34(cdc2)/cyclin B complex in vitro by human CDC25 protein. *Nature* **351**: 242-245, 1991.

Fig.1 ELISA for measurement of recombinant Chk1 and Chk2 activity using Phospho-Cdc25C Ser216 Monoclonal antibody (TK-1F1) in CycLex Checkpoint Kinase Assay/Drug Discovery Kit-1 (Cat# CY-1162)

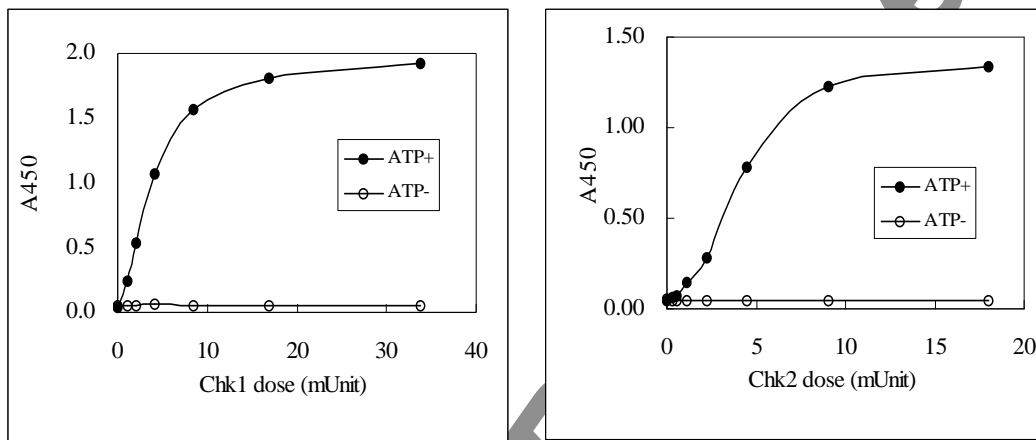
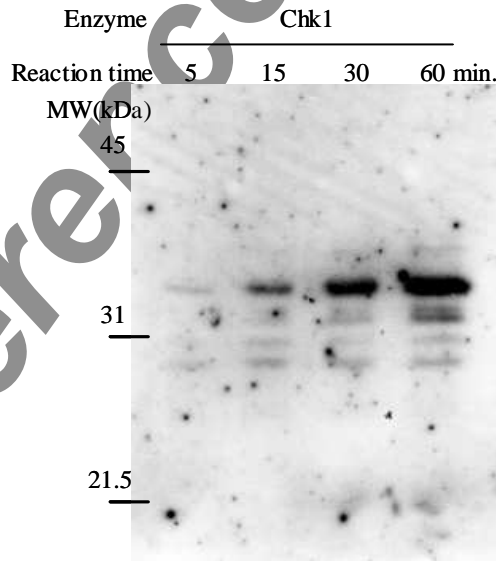


Fig.2 In vitro kinase reaction and detection of phosphorylation of Cdc25C at Ser216 residue by using Phospho-Cdc25C Ser216 Monoclonal antibody (TK-1F1). GST-Cdc25C was phosphorylated by Checkpoint kinase like as Chk1 and proved by Phospho-Cdc25C Ser216 Monoclonal antibody (TK-1F1) after SDS-PAGE and transfer to PVDF membrane.





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Western Immunoblotting Protocol

Solutions and Reagents

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

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

SDS Sample Buffer (1X): 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

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%).

10X TBS (Tris-buffered saline): 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).

Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent; 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%).

Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

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 cm² 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).
8. Electrotransfer to nitrocellulose membrane.

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 3 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 3 times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 ml of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 ml ECL™ with gentle agitation for 1 minute at room temperature.



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

Immunoprecipitation Followed by Western Immunoblotting Protocol

Solutions and Reagents

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

Cell Lysis Buffer (1X): 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na₃VO₄, 1 µg/ml Leupeptin

Note: We recommend adding 1 mM PMSF before use.

Protein A Agarose Beads: Add 5 ml of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)

3X SDS Sample Buffer: 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue,

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

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%).

10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).

Primary Antibody Dilution Buffer: 1X TBS, 0.05% Tween-20 with 5% nonfat dry milk. For 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 10 µl Tween-20 (100%).

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under non-denaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm²) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

Immunoprecipitation

1. Take 200 µL cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
2. Add Protein A Agarose Beads (20 µL of 50% bead slurry). Incubate with gentle rocking for 1-3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 2 times with 500 µL of 1X Cell Lysis Buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µL 3X SDS Sample Buffer. Vortex, then, microcentrifuge for 30 seconds.
5. Heat the sample to 95-100°C for 2-5 minutes.
6. Load the sample (15-30 µL) on SDS-PAGE gel (12-15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).



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