

Certificate of Analysis

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 800 437-7500 • F: 800 437-7502
www.millipore.com

Anti-cdk2
(rabbit polyclonal IgG)
Catalog # 07-631
Lot # 27083

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 287-298 (C-QDVTKPVPHLRL) of human cdk2, with a cysteine added for conjugation purposes.

Specificity: Recognizes human p33^{cdk2} protein kinase, Mr 33kDa.

Species Cross-reactivity: Human. Predicted to cross-react with mouse, rat, non-human primates, and hamster.

Formulation: 100µg of protein A purified rabbit IgG in 100µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

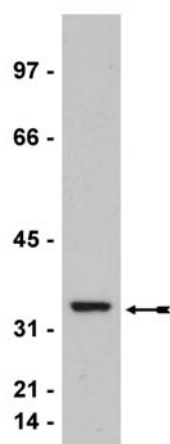
Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected cdk2 in nuclear extracts from HeLa cells.

Immunoprecipitation: 2-4µg of this lot immunoprecipitated cdk2 from 500µg of HeLa nuclear extract.

Immunoprecipitation Kinase Assay: 4µg of this lot immunoprecipitated active cdk2 from HeLa nuclear extract as shown by the ability to phosphorylate Histone H1 (Catalog # 14-155). **NOTE:** This lot also successfully immunoprecipitated cdk2 using the Catch and Release[®] v2.0 Reversible Immunoprecipitation System (Catalog # 17-500). See manual for more information and protocol:

<http://www.upstate.com/browse/productdetail.q.ProductId.e.17-500>



Immunoblot Analysis
HeLa nuclear extract was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-cdk2 (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates cdk2 (~33kDa).

General References:

1. Elledge, S.J. and M.R. Spottswood, *EMBO J.* **10**: 2653-2659, 1991.
2. Elledge, S.J., *et al.*, *Proc. Natl. Acad. Sci. USA* **89**: 2907-2911, 1992.
3. Rani, C. S., *et al.*, *J. Biol. Chem.* **272**: 10777-83, 1997.
4. Rus, H. G., *et al.*, *J. Immunol.* **156**: 4892-900, 1996.
5. Wang, X., *et al.*, *J. Biol. Chem.* **276**: 44504-44511, 2001.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a HeLa nuclear extract sample (using a modified protocol of Dignam *et al.*, Nucleic Acids Res. **22**: 1475, 1983) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS (TBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-cdk2**, diluted in freshly prepared TBS-MLK for 2 hours with agitation at room temperature.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBS-MLK for 30 minutes with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween[®]-20 for 10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Add **2-4 μ g of anti-cdk2** and 60 μ l (30 μ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500 μ l of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either PBS.
4. Dilute the cell lysate to roughly 1 μ g/ μ l total cell protein with PBS.
5. Add 500 μ g cell lysate to the reaction mixture.
6. Gently rock the reaction mixture overnight at 4°C.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer.
8. Resuspend the agarose beads in 60 μ l of 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

Immunoprecipitation Kinase Protocol

Stock Solutions:

1. **Assay Dilution Buffer I (ADBI)** (Catalog # 20-108): 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2. **Histone H1** (Catalog # 14-155): Dilute to 1mg/ml using ADBI, use 3 μ l per assay point.
3. **Magnesium/ATP Cocktail** (Catalog # 20-113): 20mM MOPS, pH 7.2, 25mM β -glycerophosphate, 5mM EGTA, 1mM Na₃VO₄, 1mM dithiothreitol, 75mM MgCl₂ and 0.5mM ATP.

Assay Procedure:

1. Add **4 μ g of anti-cdk2** and 60 μ l (30 μ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500 μ l of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold PBS.
4. Dilute the cell lysate to 1 μ g/ μ l total cell protein with PBS.
5. Add 500 μ g cell lysate to the antibody-Protein A agarose mixture.
6. Gently rock the reaction mixture overnight at 4°C.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer.
8. Add 37 μ l of ADBI (Catalog # 20-108).
9. Add 3 μ l of a 1mg/ml solution of Histone H1 (Catalog # 14-155).
10. Add 10 μ l of Magnesium/ATP cocktail (Catalog # 20-113).
11. Incubate for 30 minutes at 30°C with constant shaking.
12. End the reaction by adding an appropriate amount of 2X reducing sample buffer and boil for 5 minutes.
13. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction containing 200ng of Histone H1.