

POLYCLONAL ANTIBODY

Anti-p27^{Kip1} pAb

Code No.	Quantity	Form
554	100 µL	Affinity Purified

BACKGROUND: Cyclin dependent kinase inhibitory protein 1 (Kip1) is a 27 kDa inducible inhibitor of cyclin-dependent kinase complexes (CKI). It acts by suppressing S-phase entry and thereby blocking cell cycle progression. Kip1 interacts strongly with D-type cyclins in complex with Cdk4 and weaklier with cyclin E/Cdk2 complex to cause G₁ arrest. Kip1 is a potent tumor suppressor for multiple epithelially derived neoplasias, and expression of Kip1 is frequently down regulated in human tumors. Kip1 levels are regulated by phosphorylation and degradation via the ubiquitin-proteasome pathway.

SOURCE: This antibody was purified from rabbit serum using an affinity chromatography. The rabbit was immunized with the recombinant mouse p27^{Kip1}.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human and mouse p27^{Kip1} (27 kDa) and degradation form on Western blotting.

APPLICATIONS:

Western blotting: 1:100-1:1,000 for chemiluminescence detection system

Immunoprecipitation: 5 µL/100 µL of cell extract from 5x10⁶ cells

Immunohistochemistry: Not recommended for paraffin embedded section.

Immunocytochemistry: 1:100

Flow cytometry: 1:100 (final concentration)

Detailed procedure is provided in the following **PROTOCOLS.**

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

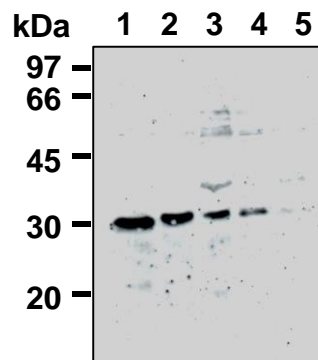
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, ZR-75-1, Hep G2, A431, MRC-5	NIH/3T3	Not tested
Reactivity on WB	+	+	

REFERENCES:

- 1) Fujita, N., *et al.*, *J. Biol. Chem.* **278**, 49254-49260 (2003)
- 2) Donovan, J. C., *et al.*, *J. Biol. Chem.* **276**, 40888-40895 (2001)
- 3) Tsutsui, T., *et al.*, *Mol. Cell Biol.* **19**, 7011-7019 (1999)
- 4) Kamesaki, H., *et al.*, *J. Immunol.* **160**, 770-777 (1998)

This antibody is used in these references.



Western blot analysis of p27^{Kip1} expression in NIH/3T3 (1), Hep G2 (2), A431 (3), ZR75-1 (4) and MRC-5 (5) using 554.

PROTOCOLS:

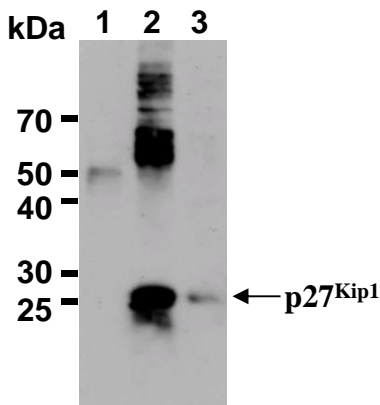
SDS-PAGE & Western Blotting

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 minutes and centrifuge. Load 20 µL

of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.

- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (5 minutes x 6 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, ZR-75-1)



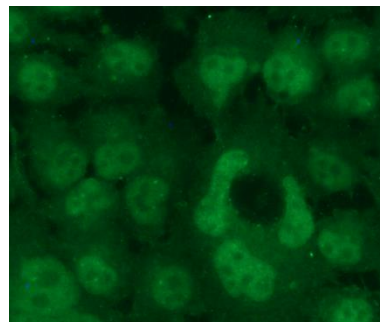
Immunoprecipitation of p27^{Kip1} from Jurkat with normal rabbit IgG (1) or 554 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-p27^{Kip1} monoclonal antibody (MBL; code no. K0082-3). Jurkat crude lysate was resolved in lane 3.

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).

- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 100 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 µL of 50% protein G agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)

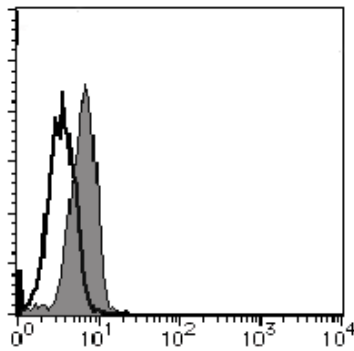


Immunocytochemical detection of p27^{Kip1} on HeLa with 554.

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10⁴ cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 20 minutes at room temperature.
- 4) The glass slide was washed with PBS 3 times.
- 5) Immerse the slide in PBS containing 0.5% Tween-20 for 10 minutes at room temperature.
- 6) The glass slide was washed 3 times with PBS.
- 7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
- 8) The glass slide was washed 3 times with PBS.
- 9) Add 50 µL of 1:100 anti-IgG (Rabbit) pAb-FITC (MBL; code no. IM-0833) diluted with PBS containing 5% normal goat serum onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) The glass slide was washed 3 times with PBS.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)



Flow cytometric analysis of p27^{Kip1} expression in Jurkat. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of 554 to the cells.

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃].
- 2) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
- 3) Wash the cells 3 times with the washing buffer.
- 4) Add 200 µL of 70% ethanol to the cell pellet after tapping. Mix well and permeabilize the cells for 30 minutes at -20°C.
- 5) Wash the cells 3 times with the washing buffer.
- 6) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 7) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Add 30 µL of 1:100 anti-IgG (Rabbit) pAb-FITC (MBL; code no. IM-0833) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; Jurkat, NIH/3T3)

RELATED PRODUCTS:

Antibody

M124-3	Anti-p15 ^{INK4b} (Human) mAb (1F6)
K0079-3	Anti-p18 ^{INK4c} (Human) mAb (DCS-118)
K0080-3	Anti-p19 ^{INK4d} (Human) mAb (DCS-100)
K0081-3	Anti-p21 ^{WAF/CIP1} (Human) mAb (DCS-60)
554	Anti-p27 ^{Kip1} pAb (polyclonal)
K0082-3	Anti-p27 ^{Kip2} mAb (DCS-72)
K0083-3	Anti-p57 ^{Kip2} (Human) mAb (DCS-230)
K0084-3	Anti-p14 ^{ARF} (Human) mAb (DCS-240)
K0162-3	Anti-Cyclin A mAb (E23.1)
K0163-3	Anti-Cyclin A mAb (E67.1)
K0163-6	Anti-Cyclin A mAb-Biotin (E67.1)
K0128-3	Anti-Cyclin B1 mAb (V152)
K0164-3	Anti-Cyclin B1 mAb (V92.1)
K0189-3	Anti-Cyclin B2 (Xenopus) mAb (X121.10)
553	Anti-Cyclin D1 pAb (polyclonal)
MD-17-3	Anti-Cyclin D1 mAb (5D4)
K0062-3	Anti-Cyclin D1 mAb (DCS-6)
K0063-3	Anti-Cyclin D2 mAb (DCS-3)
K0064-3	Anti-Cyclin D2 mAb (DCS-5)
K0013-3	Anti-Cyclin D3 mAb (DCS-22)
K0172-3	Anti-Cyclin E (Human) mAb (HE12)
K0173-3	Anti-Cyclin E (Human) mAb (HE172)
MT-19-3	Anti-Cdc2 mAb (5F6)
K0069-3	Anti-CDC6 mAb (DCS-180)
K0070-3	Anti-CDC7 (Human) mAb (DCS-342)
CY-M1021	Anti-Phospho-Cdc7 (Thr376) mAb (TK-3H7)
K0140-3	Anti-Cdc20 (Human) mAb (AR12)
K0071-3	Anti-CDC25A mAb (DCS-120)
K0072-3	Anti-CDC25A mAb (DCS-121)
K0073-3	Anti-CDC25A mAb (DCS-124)
K0075-3	Anti-CDC25C mAb (DCS-193)
K0200-1	Anti-CDC25C (Human) mAb (TC14)
CY-M1018	Anti-Phospho-Cdc25C (Ser216) mAb (TK-1F1)
K0141-3	Anti-CDC27 (Human) mAb (AF3.1)
K0150-3	Anti-CDCP1 (Human) mAb (CUB1)
K0150-4	Anti-CDCP1 (Human) mAb-FITC (CUB1)
MK-13-3	Anti-Cdk2 mAb (8A12)
K0065-3	Anti-Cdk4 mAb (DCS-156)
K0066-3	Anti-Cdk6 mAb (DCS-83)
K0067-3	Anti-Cdk6 mAb (DCS-130)
K0068-3	Anti-Cdk7 mAb (MO1)
K0085-3	Anti-Cdh1 (Fzr) mAb (DCS-266)
K0086-3	Anti-Chk1 mAb (DCS-310)
D313-3	Anti-Phospho-Chk1 (Ser296) mAb (#44-10-8)
D324-3	Anti-Phospho-Chk1 (Ser345) mAb (#83-4-43)
K0087-3	Anti-Chk2 (Human) mAb (DCS-270)
K0088-3	Anti-Chk2 (Human) mAb (DCS-273)
K0094-3	Anti-E2F-4 mAb (TFE42)
D246-3	Anti-Phospho-E2F1 (Ser364) mAb (#2)
K0095-3	Anti-DP-1 mAb (TFD10)
M043-3	Anti-DJ-1 (Human) mAb (3E8)
M069-3	Anti-MCM2 (Human) mAb (4B8)
M038-3	Anti-MCM3 (Human) mAb (3A2)
K0076-3	Anti-MCM7 mAb (DSC-141)
M049-3	Anti-MCM7 mAb (4B4)
M050-3	Anti-RCC1 mAb (3D11)
K0181-3	Anti-p53 (Human) mAb (DO-1)

K0059-3	Anti-Phospho-p53 (Ser315) (Human) mAb (FPS315)
K0060-3	Anti-Phospho-p53 (Ser392) (Human) mAb (FPS392)
CY-M1022	Anti-Phospho-p53 Ser46 mAb (TK-4D4)
CY-M1029	Anti-Acetylated Histone/p53 (Lys382) mAb (TM-5C5)
D244-3	Anti-Acetylated-p53 (Lys120) mAb (10E5)
D243-3	Anti-Acetylated-p53 (Lys382) mAb (2B7E4)
D241-3	Anti-Phospho-p53 (Ser20) mAb (17B6)
D242-3	Anti-Phospho-p53 (Ser315) mAb (#18)
D240-3	Anti-Phospho-p53 (Ser46) mAb (#36)
D245-3	Anti-Phospho-c-myc (Ser62) mAb (33A12E10)
D247-3	Anti-Phospho-MdmX (Ser367) mAb (#15)
MK-15-3	Anti-Rb (Human) mAb (3H9)
M045-3	Anti-Phospho-RB (Ser780) (Human) mAb (2C4)
555	Anti-Phospho-RB (Ser780) pAb (Polyclonal)
CY-M1013	Anti-Phospho-Rb (Ser612) mAb (3C11)
CY-M1012	Anti-Phospho-Rb (Ser612) mAb (4E4)
CY-M1015	Anti-Phospho-Rb (Ser807) mAb (5H12)
CY-M1014	Anti-Phospho-Rb (Thr356) mAb (4E3)
D248-3	Anti-Phospho-Rb (Ser795) mAb (28B5)
D249-3	Anti-Phospho-Rb (Thr821) mAb (24A7)
K0091-3	Anti-RB2 (p130) (Human) mAb (DCS-211)
D081-1	Anti-DNA Topoisomerase II α (Human) mAb (8D2)
M042-3	Anti-DNA Topoisomerase II α (Human) mAb (1C5)
M025-3	Anti-Phospho-DNA Topoisomerase II α (Thr1342) (Human) mAb (3D4)
M052-3	Anti-DNA Topoisomerase II α,β mAb (AK5)
M055-3	Anti-ORC2 (Human) mAb (3B7)
M057-3	Anti-GAK mAb (1C2)
M019-3	Anti-Nucleolin mAb (4E2)
PM026	Anti-ATM (Human) pAb (polyclonal)
M131-3	Anti-ATM (Human) mAb (4H1)
M123-3	Anti-ATR (Human) mAb (4D7)

Kits

CY-1352	CycLex [®] Cdc25A Protein Phosphatase Fluorometric Assay Kit
CY-E1352	Recombinant Cdc25A (Catalytic Domain)
CY-1353	CycLex [®] Cdc25B Protein Phosphatase Fluorometric Assay Kit
CY-E1353	Recombinant Cdc25B (Catalytic Domain)
CY-1354	CycLex [®] Cdc25C Protein Phosphatase Fluorometric Assay Kit
CY-E1354	Recombinant Cdc25C (Catalytic Domain)
CY-1355	CycLex [®] Cdc25 Combo Protein Phosphatase Fluorometric Assay Kit
CY-7049	CycLex [®] Total p53 ELISA Kit
CY-7050	CycLex [®] Phospho-p53 Ser46 ELISA Kit
CY-7051	CycLex [®] Phospho-p53 Ser392 ELISA Kit