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

MONOCLONAL ANTIBODY

Anti-Rb

Code No.	Clone	Subclass	Quantity	Concentration
MK-15-3	3H9	Mouse IgG2a κ	100 μg	1 mg/mL

BACKGROUND: Mutation of the retinoblastoma tumor suppressor gene alone is sufficient to cause retinoblastoma in humans, suggesting that it might play a role in the normal coordination of cell proliferation and cell death. Deletion or mutational inactivation of the retinoblastoma tumor suppressor protein (Rb) is correlated with the genesis of a variety of human cancers including retinoblastoma, osteosarcoma, and carcinomas of the breast, bladder, and lung. Rb protein is phosphorylated by cyclin D-Cdk4/Cdk6 and cyclin A/cyclin E-Cdk2 during the G_1/S transition. This phosphorylation causes the inactivation of the growth inhibitory functions of Rb. Rb undergo phosphorylation and attendant functional inactivation, the cell proceed into late G_1.

SOURCE: This antibody was purified from hybridoma (clone 3H9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human Rb protein (612-928 aa).

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

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

REACTIVITY: This antibody reacts with human retinoblastoma gene product (110-115 kDa).

APPLICATIONS:

Western blotting; 5-10 μg/mL for chemiluminescence detection system
Immunoprecipitation; 1-5 μg/200-300 μL of cell extract
Immunohistochemistry; 5 μg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji, HL-60	WR19L	PC12
Reactivity on WB	+	-	-

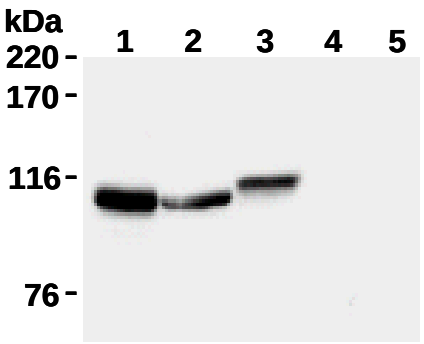
INTENDED USE:

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

REFERENCES:

1) Haraguchi, T., *et al., J. Cell Sci.* **120**, 1967-1977 (2007)
2) Tokugawa, T., *et al., Cancer Res.* **62**, 4938-4944 (2002)
3) Nishio, M., *et al., Clin. Cancer Res.* **3**, 1051-1058 (1997)
4) Lin, B. T., *et al., EMBO J.* **10**, 857-864 (1991)
5) Wang, N. P., *et al., Cell Growth Differ.* **1**, 233-239 (1990)
6) Taya, Y., *et al., Biochem. Biophis. Res. Communu.* **164**, 580-586 (1989)
7) Lee, W. H., *et al., Science* **235**, 1394-1399 (1987)
8) Lalande, M., *et al., Cancer Genet. Cytogenet.* **13**, 283-295 (1984)

Clone 3H9 is used in reference number 1) - 3).



Western blot analysis of Rb expression in Jurkat (1), Raji (2), HL-60 (3), WR19L (4) and PC12 (5) using MK-15-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

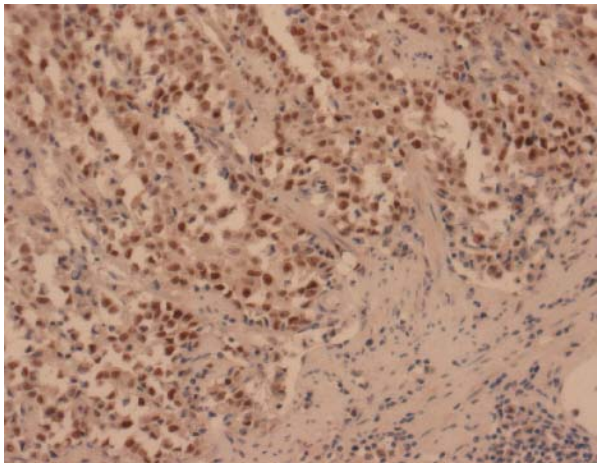
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).

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 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% 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 suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
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 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, HL-60)

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 suggest in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) 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**.)



Immunohistochemical detection of Rb on human stomach paraffin embedded section with MK-15-3.

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
Heat treatment by Microwave:
Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).

11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive controls for Immunohistochemistry; human stomach)

RELATED PRODUCTS:

MK-15-1	anti-Rb (3H9)
K0091-3	anti-Rb2 (DCS-211)
M045-3	anti-phospho Rb (Ser780) (2C4)
555	anti-phospho Rb (Ser780) (Polyclonal)
D248-3	anti-phospho Rb (Ser795) (28B5)
D249-3	anti phospho Rb (Thr821) (24A7)
K0162-3	anti-Cyclin A (E23.1)
K0163-3	anti-Cyclin A (E67.1)
K0163-6	Biotin labeled anti-Cyclin A (E67.1)
K0128-3	anti-Cyclin B1 (V152)
K0164-3	anti-Cyclin B1 (V92.1)
K0189-3	anti-Cyclin B2 (X121.10)
553	anti-Cyclin D1 (polyclonal)
MD-17-3	anti-Cyclin D1 (5D4)
MD-17-3H	anti-Cyclin D1 (5D4)
K0062-3	anti-Cyclin D1 (DCS-6)
K0063-3	anti-Cyclin D2 (DCS-3)
K0064-3	anti-Cyclin D2 (DCS-5)
K0013-3	anti-Cyclin D3 (DCS-22)
K0172-3	anti-Cyclin E (HE12)
K0173-3	anti-Cyclin E (HE172)
MT-19-3	anti-Cdc2Hs (5F6)
K0069-3	anti-CDC6 (DCS-180)
K0070-3	anti-CDC7 (DCS-342)
CY-M1021	anti-Phospho-Cdc7 Thr376 (TK-3H7)
K0140-3	anti-Cdc20 (AR12)
K0071-3	anti-CDC25A (DCS-120)
K0072-3	anti-CDC25A (DCS-121)
K0073-3	anti-CDC25A (DCS-124)
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)
K0075-3	anti-CDC25C (DCS-193)
K0200-3	anti-Cdc25C (TC14)
CY-M1018	anti-Phospho-Cdc25C Ser216 (TK-1F1)
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
K0141-3	anti-CDC27 (AF3.1)
K0150-3	anti-CDCP1 (CUB1)
K0150-4	FITC labeled anti-CDCP1 (CUB1)
MK-13-3	anti-Cdk2 (8A12)
K0065-3	anti-Cdk4 (DCS-156)
K0066-3	anti-Cdk6 (DCS-83)
K0067-3	anti-Cdk6 (DCS-130)
K0068-3	anti-Cdk7 (DCS-MO1)
K0077-3	anti-$p16^{INK4a}$ (DCS-50)
M124-3	anti-$p15^{INK4b}$ (1F6)
K0079-3	anti-$p18^{INK4c}$ (DCS-118)
K0080-3	anti-$p19^{INK4d}$ (DCS-100)
K0081-3	anti-$p21^{WAF/CIP1}$ (DCS-60)
K0082-3	anti-$p27^{Kip2}$ (DCS-72)
K0083-3	anti-$p57^{Kip2}$ (DCS-230)
K0084-3	anti-$p14^{ARF}$ (DCS-240)
K0085-3	anti-Cdh1 (DCS-266)
K0086-3	anti-Chk1 (DCS-310)
K0087-3	anti-Chk2 (DCS-270)
K0088-3	anti-Chk2 (DCS-273)
K0094-3	anti-E2F-4 (TFE42)
K0095-3	anti-DP-1 (TFD10)
M043-3	anti-DJ-1 (3E8)
M069-3	anti-MCM2 (4B8)
M038-3	anti-MCM3 (3A2)
M049-3	anti-MCM7 (4B4)
M050-3	anti-RCC1 (3D11)
K0181-3	anti-p53 (DO-1)
D241-3	anti-phospho-p53 (Ser20) (17B6)
D240-3	anti-phospho-p53 (Ser46) (#36)
CY-M1022	anti-phospho-p53 Ser46 (TK-4D4)
D244-3	anti-acetylated p53 (Lys120) (10E5)
K0059-3	anti-phospho-p53 Ser315 (FPS315)
D243-3	anti-acetylated p53 (Lys382) (2B7E4)
K0060-3	anti-phospho-p53 Ser392 (FPS392)
D242-3	anti-phospho p53 (Ser315) (#18)
CY-M1029	anti-Acetylated Histone/p53-Lys382 (TM-5C5)
CY-7049	CycLex® Total p53 ELISA Kit
CY-7050	CycLex® Phospho-p53 Ser46 ELISA Kit
CY-7051	CycLex® Phospho-p53 Ser392 ELISA Kit
D245-3	anti-phospho c-Myc (Ser62) (33A12E10)
D246-3	anti-phospho E2F-1 (Ser364) (#2)
D247-3	anti-phospho Mdmx (Ser367) (#15)
D081-1	anti-DNA Topoisomerase IIα (8D2)
M025-3	anti-phospho DNA Topoisomerase IIα (3D4)
M052-3	anti-DNA Topoisomerase II αβ (AK5)
M055-3	anti-ORC2 (3B7)
M057-3	anti-GAK (1C2)
M019-3	anti-Nucleolin (4E2)
PM006-3	anti-phospho Histone H3 (Polyclonal)
M123-3	anti-ATR (4D7)
M131-3	anti-ATM (4H1)
PM026	anti-ATM (polyclonal)