

MONOCLONAL ANTIBODY

# Anti-Phospho RB (Ser780)

Code No.	Clone	Subclass	Quantity	Concentration
M045-3	2C4	Mouse IgG1	100 µg	1 mg/mL

**BACKGROUND:** Cyclin-dependent kinases (Cdk) play important roles in the regulation of the cell cycle. RB protein (pRB) is phosphorylated by cyclin D-Cdk4/Cdk6 and cyclin A/cyclin E-Cdk2 during the G<sub>1</sub>/S transition. This phosphorylation causes the inactivation of the growth inhibitory functions of pRB. pRB undergo phosphorylation and attendant functional inactivation, the cell proceed into late G<sub>1</sub>. Cyclin D-Cdk4 specifically phosphorylates Ser780 in pRB, while cyclin E-Cdk2 and cyclin A-Cdk2 does not.

**SOURCE:** This antibody was purified from hybridoma (clone 2C4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with synthetic human phospho-RB (Ser780) peptide, TRPPTLS (PO3) PIPHIP, which corresponding to amino acids 774-786 of human pRB.

**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.

**SPECIFICITY:** This antibody recognizes only phosphorylation site-sequence, RPPTLS (PO3) PIPHIPR. The antibody detects 115 kDa of human phospho-RB on Western blotting.

**APPLICATIONS:**

- Western blotting: 1 µg/mL for chemiluminescence detection system
- Immunoprecipitation: Not tested
- Immunohistochemistry: Not tested
- Immunocytochemistry: Not tested
- Flow cytometry: Not tested

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

**INTENDED USE:**

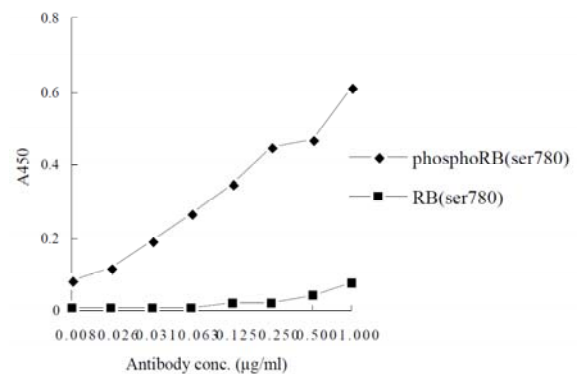
For Research Use Only. Not for use in diagnostic procedures.

**SPECIES CROSS REACTIVITY:**

Species	Human		Mouse	Rat
Cells	Jurkat, U937, MOLT4	5637*, Saos2**	WR1-Rb-1, NIH/3T3, Ba/F3	Not Tested
Reactivity on WB	+	-	-	

\*5637: ATCC HTB9 (RB mRNA negative)  
\*\*Saos2: ATCC HTB85 (RB mRNA negative)

**ELISA TEST:** The reactivity of Anti-phospho-RB (Ser780) against synthetic human phospho-RB peptide and nonphospho-RB peptide.



**REFERENCES:**

- 1) Suzui, M., *et al.*, *Cancer Res.* **62**, 3997-4006 (2002)
- 2) Kitagawa, M., *et al.*, *EMBO J.* **15**, 7060-7069 (1996)
- 3) Weinberg, R. A., *Cell* **81**, 323-330 (1995)

This antibody is used in reference number 1).

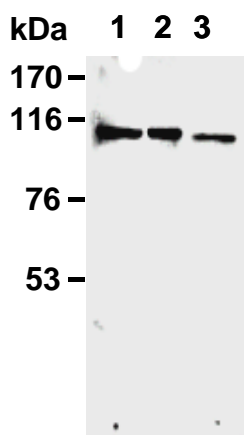
**PROTOCOL:**

**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  $\mu$ L 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<sup>2</sup> 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 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 5% 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 1:5,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 5% 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. 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, U937, MOLT4)



**Western blot analysis of Phospho RB (Ser780) expression in Jurkat (1), MOLT4 (2) and U937 (3) using M045-3.**

**RELATED PRODUCTS:**

- 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)
- K0140-3 Anti-Cdc20 (AR12)
- K0071-3 Anti-CDC25A (DCS-120)
- K0072-3 Anti-CDC25A (DCS-121)
- K0073-3 Anti-CDC25A (DCS-124)
- 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-E1354 Recombinant Cdc25C (Catalytic Domain)
- 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<sup>INK4a</sup> (DCS-50)
- M124-3 Anti-p15<sup>INK4b</sup> (1F6)
- K0079-3 Anti-p18<sup>INK4c</sup> (DCS-118)
- K0080-3 Anti-p19<sup>INK4d</sup> (DCS-100)
- K0081-3 Anti-p21<sup>WAF/CIP1</sup> (DCS-60)
- K0082-3 Anti-p27<sup>Kip2</sup> (DCS-72)
- K0083-3 Anti-p57<sup>Kip2</sup> (DCS-230)
- K0084-3 Anti-p14<sup>ARF</sup> (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)
- MK-15-1 Anti-RB (3H9)
- 555 Anti-Phospho RB (Ser 780) (Poly)
- K0091-3 Anti-RB2 (DCS-211)
- M025-3 Anti-Phospho DNA Topoisomerase II $\alpha$  (3D4)
- M052-3 Anti-DNA Topoisomerase II  $\alpha\beta$  (AK5)
- M055-3 Anti-ORC2 (3B7)
- M057-3 Anti-GAK (1C2)
- M019-3 Anti-Nucleolin (4E2)
- PM006-3 Anti-Phospho Histone H3 (Poly)
- PM026 Anti-ATM (polyclonal)