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POLYCLONAL ANTIBODY

Anti-rck (p54) pAb

Code No.	Quantity	Form
PD009	100 µL	Affinity Purified

BACKGROUND: The lymphoma-associated rck (p54) gene product is a member of the DEAD box protein/RNA helicase family which has a variety of functions such as translation initiation, pre-mRNA splicing and ribosome assembly. Overexpression of human rck in a guinea pig cell line strongly inhibited the cell growth, and rck (p54) overexpression was found in >65% of colorectal tumours and >90% of hepatocellular carcinomas. Expression in tumors highly correlates with c-myc expression (>92%), suggesting rck (p54) may contribute to cell proliferation and carcinogenesis by increasing synthesis of c-myc protein via increased translation initiation of c-myc mRNA.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with KLH conjugated synthetic peptide KLH- STARTENPVIC.

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 rck (p54) (54 kDa) on Western blotting and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; 1:100
Immunocytochemistry; Reference 2), 6), 8)-9) and 11).
Flow Cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HL-60, Jurkat	WR19L, NIH/3T3	PC12
Reactivity on WB	+	+	+

INTENDED USE:

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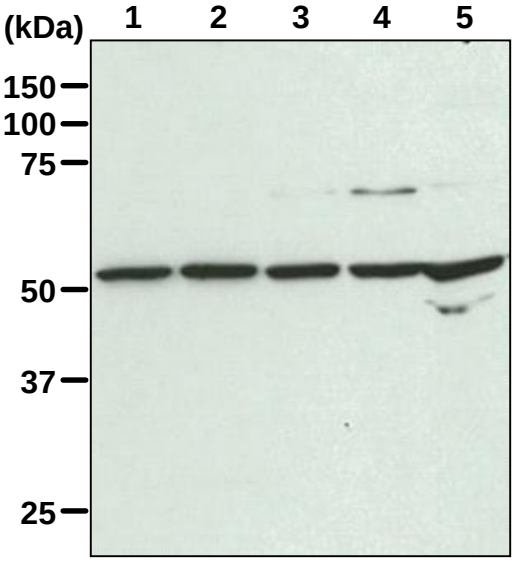


Western blot analysis of rck (p54)
Lane 1: HL-60 Lane 4: NIH/3T3
Lane 2: Jurkat Lane 5: PC12
Lane 3: WR19L
Immunoblot with PD009

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

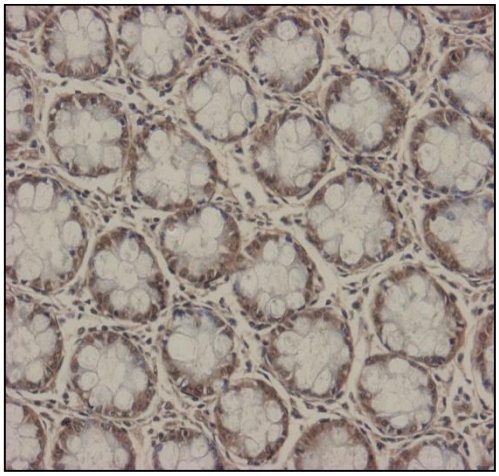
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 seconds).

2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) 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.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 minutes x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) 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.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) 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.
11) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HL-60, Jurkat, WR19L, NIH/3T3, PC12)



Immunohistochemical detection of rck (p54)
Human colon cancer
Immunohistochemical staining with PD009

Immunohistochemical staining for paraffin-embedded sections

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) Cover each section with 3% H_2O_2 in PBS for 10 minutes at room temperature to block endogenous peroxidase activity.
5) Wash the slides with PBS 2 times for 3-5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES (pH 7.2), 1% BSA, 135 mM NaCl) 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 blocking buffer as suggest in the **APPLICATIONS**. Incubate the sections for 1 hour at room temperature.
8) Wash the slides with PBS 2 times for 3-5 minutes each.
9) Wipe gently around each section and cover tissues with Histostar™ (rabbit) (MBL; code no. 8466). Incubate for 1 hour at room temperature.
10) Wash the slides with PBS 2 times for 3-5 minutes each.
11) Visualize by reacting for 10 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides with PBS 2 times for 3-5 minutes each.
13) 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.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Human colon cancer)

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