POLYCLONAL ANTIBODY

**Anti-rck (p54) pAb**

**Code No.**
PD009

**Quantity**
100 μL

**Form**
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

**SPECIES CROSS REACTIVITY:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>HL-60, Jurkat</td>
<td>WR19L, NIH/3T3</td>
<td>PC12</td>
</tr>
<tr>
<td>Reactivity on WB</td>
<td>+</td>
<td>+</td>
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**INTENDED USE:**
For research use only. Not for clinical diagnosis.

**REFERENCES:**

**PROTOCOLS:**

**SDS-PAGE & Western Blotting**
1. Wash 1 x 10⁷ 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.
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.

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 from 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)

Immunochemochemical 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% H₂O₂ 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)

**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Antibody Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN06M</td>
<td>Anti-EIF4E mAb (C107-3-5)</td>
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<tr>
<td>RN01P</td>
<td>Anti-EIF4E pAb</td>
</tr>
<tr>
<td>RN004M</td>
<td>Anti-Ribosomal P0/P1/P2 mAb (9D5)</td>
</tr>
<tr>
<td>RN028P</td>
<td>Anti-EIF2C1 (AGO1) pAb</td>
</tr>
<tr>
<td>RN028PW</td>
<td>Anti-EIF2C1 (AGO1) pAb</td>
</tr>
<tr>
<td>RN003M</td>
<td>Anti-EIF2C2 (AGO2) mAb (1B1-E2H5)</td>
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<tr>
<td>RN005M</td>
<td>Anti-EIF2C2 (AGO2) mAb (2A8)</td>
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<tr>
<td>RN029PW</td>
<td>Anti-EIF2C2 (AGO2) pAb</td>
</tr>
<tr>
<td>RN031PW</td>
<td>Anti-ZFIP36 (Human) pAb</td>
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<tr>
<td>RN033P</td>
<td>Anti-TNRC6A (Human) pAb</td>
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<tr>
<td>RN038P</td>
<td>Anti-CPEB1 pAb</td>
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<tr>
<td>RN049PW</td>
<td>Anti-G3BP2 pAb</td>
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<td>RN086PW</td>
<td>Anti-U2AF2 pAb</td>
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<tr>
<td>RN100PW</td>
<td>Anti-EXOSC5 (RRP46) (Human) pAb</td>
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<tr>
<td>RN102PW</td>
<td>Anti-GEMIN2 (Human) pAb</td>
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<td>RN103PW</td>
<td>Anti-NCBP1 (CBP80) pAb</td>
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<td>RN104PW</td>
<td>Anti-PAN2 (USP52) (Human) pAb</td>
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<tr>
<td>RN105PW</td>
<td>Anti-PARN pAb</td>
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<tr>
<td>RN107PW</td>
<td>Anti-TARDBP (TDP-43) pAb</td>
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<td>RN108PW</td>
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<td>RN109PW</td>
<td>Anti-XRN1 (Human) pAb</td>
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<td>RN110PW</td>
<td>Anti-CNOT7 (CAF1) pAb</td>
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<tr>
<td>RN111PW</td>
<td>Anti-ETF1 (eRF1) pAb</td>
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</tbody>
</table>

Other related antibodies and kits are also available. Please visit our website at [http://ruo.mbl.co.jp/](http://ruo.mbl.co.jp/)

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