

**POLYCLONAL ANTIBODY**

# Anti-HRF/TCTP

**Code No.**  
PM017

**Quantity**  
100 µL

**Form**  
Rabbit IgG

**BACKGROUND:** The pathophysiological events of human allergic disease are characterized by infiltration of inflammatory cells and by the presence of mediators such as histamine. An IgE-dependent histamine releasing factor (HRF) produced by lymphocytes of atopic children and present in biological fluids of allergic patients has been identified and purified. HRF, also known as TCTP (Translationally Controlled Tumor Protein), Fortilin, Tumor protein-1 (Tpt-1), lens epithelial protein and p23, is a 21 or 23 kDa protein produced in a variety of mouse and human cell lines, respectively. HRF binds to a unique species of IgE on the surface of mast cells and basophils and thereby causes histamine release. The histamine released in late-phase reaction (LPR) of chronic allergic diseases such as asthma results from activation of basophils, eosinophils and lymphocytes infiltrate tissues during this response.

**SOURCE:** This antibody was purified from rabbit serum using protein A agarose. The rabbit was immunized with recombinant full length human HRF.

**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 HRF on Western blotting.

**INTENDED USE:**

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

**APPLICATIONS:**

Western blotting; 1:1,000 for chemiluminescence detection system

Immunoprecipitation; Not recommended

Immunohistochemistry; 1:1,000

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:**

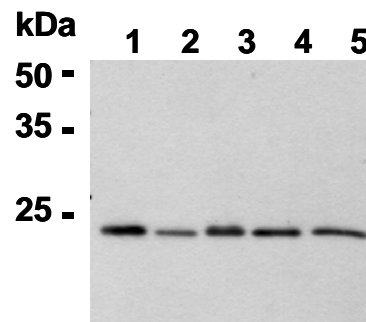
Species	Human	Mouse	Rat
Cells	U937, 293T	WR19L	PC12, Rat1
Reactivity on WB	+	+	+

**REFERENCE:**

1) Tuynder, M., *et al.*, *PNAS*. **101**, 15364-15369 (2004)

**RELATED PRODUCT:**

M099-3 Anti-HRF (6E9)



**Western blot analysis of HRF/TCTP expression in U937 cells (1), 293T cells (2), PC12 cells (3), WR19L cells (4) and Rat1 cells (5) using PM017.**

**PROTOCOLS:**

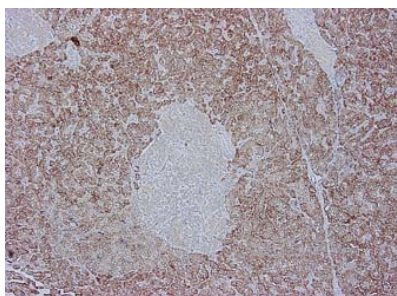
**SDS-PAGE & Western Blotting**

- 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 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 2 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<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 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 to be used will be depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
- 9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (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 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for western blotting; U937, 293T, WR19L, PC12, Rat1)



**Immunohistochemical detection of HRF/TCTP on paraffin embedded section of human pancreas PM017.**

temperature for about 40 minutes.

- 5) Remove the slides from the citrate buffer and cover each section with 3% H<sub>2</sub>O<sub>2</sub> 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; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific antibody 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 10 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 10 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<sub>2</sub>O<sub>2</sub> 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 control for immunohistochemistry; human pancreas)

#### **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 oven:

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