

**Anti-Rat Cathepsin B**  
(rabbit polyclonal IgG)

**Catalog # 06-480**

**Lot # 14927**

**Background:** Cathepsin B is a lysosomal cysteine proteinase that is important for intracellular protein catabolism. Cathepsin B has been implicated in several disease states including muscular dystrophy, rheumatoid arthritis and tumor metastasis.

**Immunogen:** Recombinant rat procathepsin B <sup>1</sup>.

**Specificity:** Recognizes procathepsin B (≈40kDa) and mature cathepsin B (25kDa, 26kDa and 30kDa).

**Species Cross-reactivity:** Mouse.

**Quantity and Formulation:** **200ng** of protein A purified rabbit IgG in **200ml** of 0.1M Tris-glycine buffer, pH 7.4 with 0.05% NaN<sub>3</sub>.

**Physical Form:** Frozen liquid.

**Storage and Shelf Life:** 2 years at -20°C. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

**References:**

1. Rowan, A. D., *et. al.*, Biol. Chem. Hoppe-Seyler **373**: 427-432, 1992.
2. Lee, E. R., *et. al.*, J. Histochem. Cytochem. **43**: 525-536, 1995.

**FOR IN VITRO RESEARCH USE ONLY.**  
**NOT FOR USE IN HUMANS OR .**

### Quality Control Testing and Research Applications

Western Immunoblot Analysis: This lot of antibody at 0.5 and 2.0 µg/ml detected both procathepsin B (≈40kDa) and cathepsin B (30kDa and 26kDa) in 20 µg of rat kidney microsomal preparation.

Immunohistochemistry<sup>2</sup>: This lot of antibody at 10 µg/ml positively stained rat kidney and liver sections that had been fixed with ethanol:acetic acid [95:5] for 5 minutes at room temperature.

#### Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a tissue lysate that has been sonicated and clarified by centrifugation at 14,000 X g and 4°C for 15 minutes (lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 µg/ml aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose in **0.5-2.0ng/ml a-Cathepsin B** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit IgG linked to horseradish peroxidase, 1:3000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

#### Immunohistochemistry

1. Fix 10µ frozen tissue sections in 95% ethanol/5% acetic acid for 5 minutes at room temperature.
2. Wash the sections with PBS for 15 minutes at room temperature.
3. Add 400µl of 8% albumin in PBS and incubate for 30 minutes at room temperature.
4. Wash the sections with PBS for 15 minutes at room temperature.
5. Incubate the sections with **10ng/ml a-Cathepsin B**, containing 1% BSA in PBS overnight at 4°C. Also run a negative control (no primary antibody) to check for non-specific staining.
6. Wash the sections with PBS for 30 minutes at room temperature.
7. Incubate the sections with a 1:100 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in PBS for 3 hours at room temperature in the dark.
8. Wash the section with PBS for 30 minutes in the dark.
9. Mount coverslips over the sections.
10. Examine the sections under a fluorescent microscope.