



cell signaling solutions

Certificate of Analysis

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Anti-Cathepsin B

(rabbit polyclonal IgG)

Catalog # 06-480

Lot # 23694

Immunogen: Recombinant rat procathepsin B ¹.

Specificity: Recognizes procathepsin B, Mr 40kDa and mature cathepsin B, Mr 25kDa, 26kDa and 30kDa.

Species Cross-reactivity: Mouse.

Formulation: 400µg packaged as two vials, each vial containing 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. 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.

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

Quality Control Testing and Research Applications

Immunoblot Analysis: 2-4µg/ml of this lot of antibody detected both procathepsin B and cathepsin B in 20µg of rat kidney microsomal preparation (Catalog # 12-146).

Immunohistochemistry²: A previous lot of this antibody at 10µg/ml positively stained rat kidney and liver sections that had been fixed with ethanol:acetic acid [95:5] for five minutes at room temperature.

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.

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; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 (Catalog # 20-200), (PBS-MLK) for 20-60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose in **2-4µg/ml anti-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 HRP conjugated IgG, Catalog # 12-348, 1:5000 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 **10µg/ml anti-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.