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cell signaling solutions
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Certificate of Analysis

Anti-Cathepsin B

(rabbit polyclonal IgG)
Catalog # 06-480
Lot # 1350293

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.

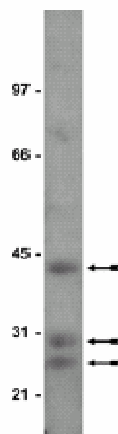
Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

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).

Immunohistochemistry[2]: 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.



Immunoblot Analysis
Rat kidney microsomal preparation was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-cathepsin B (4µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Top arrow indicates procathepsin B (~40kDa). Two lower arrows indicate mature cathepsin B (~25 & 30kDa).

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℃ f or 15 minutes (lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1_g/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 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), (PBSMLK) for 20-60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose in **2-4_g/ml anti-Cathepsin B** diluted in freshly prepared PBS-MLK overnight with agitation at 4℃.
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 10_ 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 400_l 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 **10_g/ml anti-Cathepsin B**, containing 1% BSA in PBS overnight at 4℃. Also r un 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.