



Certificate of Analysis

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## Anti-Human Cathepsin D

(rabbit polyclonal IgG)

Catalog # 06-467

Lot # 14116

**Immunogen:** Active cathepsin D (46kDa) isolated from human liver.

**Specificity:** Recognizes both active cathepsin D and its precursor at 54kDa.

**Species Cross Reactivity:** Human

**Formulation:** 200 $\mu$ g of protein A-purified rabbit IgG in 166 $\mu$ l of 0.1M Tris-glycine, pH 7.4. Frozen solution.

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

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

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### Quality Control Testing

**Immunoblot Analysis:** Used at 4 $\mu$ g/ml, this lot of antibody detected the precursor of cathepsin D in human A431 cells

**Included Positive Antigen Control for Immunoblot**

**Analysis:** Catalog # 12-301, human A431 cell lysate. Use 20 $\mu$ g per lane for minigels.

### Additional Research Applications

**Immunocytochemistry:** Recommended for immunocytochemistry as demonstrated by an independent laboratory.

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**Background:** Cathepsin D is a lysosomal enzyme involved in intracellular protein turnover. It is synthesized as an inactive 54kDa precursor and proteolytically processed to an active form of approximately 46kDa. While the precursor has mitogenic activity, the active form of the enzyme appears closely associated with proteolysis of proteoglycan and collagen of cartilage, with bone resorption, as well as with the possible activation of several transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ).

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/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, 0.05% Tween 20 (PBS-MLK-T) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose in **4 $\mu$ g/ml of the  $\alpha$ -human cathepsin D** 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).