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## Certificate of Analysis

### Anti-Cathepsin D

(rabbit polyclonal IgG)

Catalog # 06-467

Lot # 23715

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

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

**Species Cross Reactivity:** Human.

**Formulation:** 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.1mM EDTA, and 0.05% sodium azide. Frozen liquid.

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

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

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### Quality Control Testing

Immunoblot Analysis: 4µg/ml of this lot detected the Cathepsin D and its precursor in human A431 cells.

**Included Positive Antigen Control for Immunoblot Analysis:** Catalog # 12-301, Non-Stimulated A431 Cell Lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

### Additional Research Applications

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

### 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; 1µg/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. Wash blot for 10 minutes in PBS with 0.05% Tween 20, and rinse with water.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (MLK), Catalog # 20-200, for 1 hour at 4°C with constant agitation.
4. Incubate the nitrocellulose in **4µg/ml of the anti-cathespain D** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. 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.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).