

#### ORDERING INFORMATION

Catalog Number: AF1202

Lot Number: JWH01

**Size:** 100 µg

Formulation: 0.2 μm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human Cystatin D

Immunogen: NS0-derived rhCystatin D (aa 28 - 142)

Ig Type: human Cystatin D specific goat IgG

Applications: Neutralization Direct ELISA Western blot

# Anti-human Cystatin D Antibody

### Preparation

Produced in goats immunized with purified, NS0-derived, recombinant human Cystatin D (rhCystatin D; aa 28 - 142). Human Cystatin D specific IgG was purified by human Cystatin D affinity chromatography.

#### Formulation

Lyophilized from a 0.2  $\mu m$  filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

#### Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 0.1 mg/mL.

#### Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.** 

# **Specificity**

This antibody has been selected for its ability to recognize human Cystatin D in direct ELISAs and western blots. In these formats, this antibody shows approximately 10% cross-reactivity with rhCystatin C, rhCystatin SN and rhCystatin S and less than 1% cross-reactivity with rhCystatin E/M and rhFetuin A.

# Applications

**Neutralization** - This antibody has been used to neutralize the inhibitory activity of rhCystatin D (R&D Systems, Catalog # 1202-PI) against papain. The antibody was preincubated with the inhibitor at different molar ratios at room temperature for 30 minutes. The resulting mixtures were then incubated with the enzyme at room temperature for 15 minutes. The enzyme was assayed with a peptide substrate (R&D Systems, Catalog # ES009). 50% of the proteolytic activity was restored by the antibody at 13 µg/mL (IC<sub>50</sub>) under conditions in which 1 µg/mL of the inhibitor, 0.1 µg/mL of the enzyme and 100 µM of the substrate were present. Considering the molecular masses of the inhibitor (15 kDa) and the antibody (150 kDa), IC<sub>50</sub> was achieved at approximately 1:1 molar ratio of the antibody to the inhibitor.

**Direct ELISA -** This antibody can be used at 0.5 - 1.0  $\mu$ g/mL with the appropriate secondary reagents to detect human Cystatin D. The detection limit for rhCystatin D is approximately 0.3 ng/well.

**Western blot -** This antibody can be used at 0.1 - 0.2  $\mu$ g/mL with the appropriate secondary reagents to detect human Cystatin D. The detection limit for rhCystatin D is approximately 2 ng/lane under non-reducing and reducing conditions.

Optimal dilutions should be determined by each laboratory for each application.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.