Affinity-Purified Sheep Anti-human/mouse/rat PDCD6 Antibody

ORDERING INFORMATION

Catalog Number: AF6015

Lot Number: CDLN03

Size: 100 µg

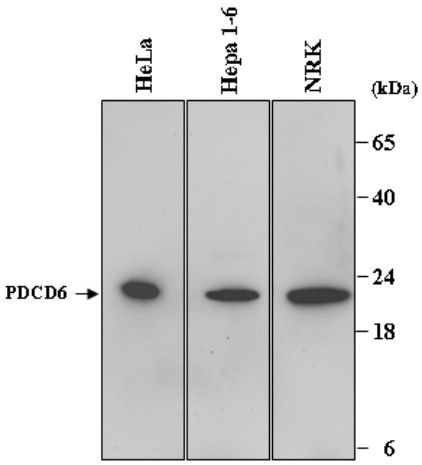
Storage: -20° C

Specificity: human, mouse and rat PDCD6

Immunogen: *E. coli* derived recombinant human PDCD6 (aa 1 - 189)

Ig Type: sheep IgG

Application: Western blot



Detection of PDCD6 with AF6015.
Lysates from human HeLa, mouse Hepa 1-6 and rat NRK cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 µg/mL sheep anti-PDCD6 as described in *Protocols for Immunoblotting*.

Background

PDCD6 (Programmed cell death protein 6; also apoptosis-linked gene 2 protein, ALG-2) is a 22 kDa member of the penta-EF hand protein family of molecules. It is ubiquitously expressed, and appears to perform multiple functions. PDCD6 is functionally linked to apoptosis, proliferation, membrane fusion and vesicle trafficking. Human PDCD6 is 191 amino acids (aa) in length and although it contains five EF-hand domains, only domains 1 and 3 are considered functional (aa 23 - 58 and 90 - 125). There is one splice form (ALG-2.1) that shows a deletion of Gly121Phe122. This form typically represents about 1/3 of total cellular PDCD6 and may affect the $C2^{2+}$ binding properties of ALG-2.1. PDCD6/ALG-2 will homodimerize, and heterodimerize with ALG-2.1. Notably, there are classes of cytosolic proteins that bind PDCD6, but not ALG-2.1, accounting for differences in function. Both human PCD6 isoforms are 99% aa identical to their mouse and rat counterparts.

Preparation

Produced in sheep immunized with purified, *E. coli* derived, recombinant human Programmed Cell Death protein 6 (PDCD6; aa 1 - 189; Accession # O75340). Human PDCD6 specific IgG was purified by affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN_3. The antibody concentration will be 1.0 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

The antibody detects endogenous human, mouse and rat PDCD6 at ~22 kDa by Western blot.

Application

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in antibody solution containing 1.0 µg/mL sheep anti-human/mouse/rat PDCD6.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated Donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with chemiluminescent reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3 - 4 bursts of 5 - 10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

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

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