Human/Mouse/Rat ATG3 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF5450

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects endogenous human, mouse and rat ATG3 in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human ATG3 Met1-His287 Accession # Q9NT62
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

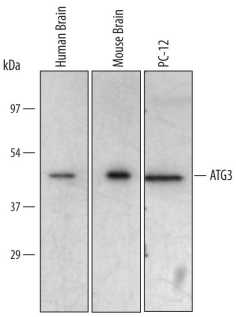
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

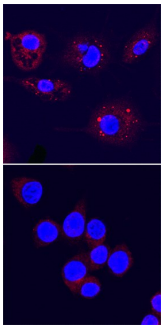
DATA

Western Blot



Detection of Human/Mouse/Rat ATG3 by Western Blot. Western blot shows lysates of human brain tissue, mouse brain tissue, and PC-12 rat adrenal pheochromocytoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat ATG3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5450) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ATG3 at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Immunocytochemistry



ATG3 in RAW 264.7 Mouse Cell Line. ATG3 was detected in immersion fixed RAW 264.7 mouse monocyte/macrophage cell line, untreated (lower panel) or treated with LPS (upper panel), using Sheep Anti-Human/Mouse/Rat ATG3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5450) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to autophagosomes. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

ATG3 (autophagy-related protein 3; also APG3-like and PC3-96) is a ubiquitous 45 kDa member of the ATG3 family of proteins. It functions as an E2-like enzyme during the initial stages of autophagosome formation by catalyzing the transfer of ATG7-bound ATG8 (known as LC3, GATE16 and GABA-RAP in mammals) to phosphatidylethanolamine, critical for autophagy. Human ATG3 is 314 amino acids in length and contains an active site at Cys264 that forms a thiol ester bond with the C-terminal Gly of ATG8. There are multiple potential isoform variants. Three show a 22, 28 and 35 aa substitution for aa 289-314, respectively, while a fourth shows an alternate start site at Met88 that may be accompanied by one of the afore mentioned substitutions. Over aa 1-287, human ATG3 shares 97.9% aa identity with mouse ATG3.

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