

DESCRIPTION

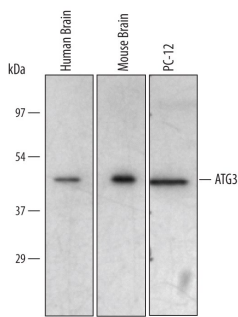
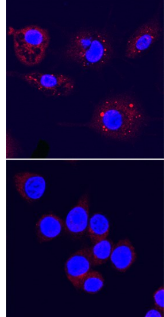
Species Reactivity	Human/Mouse/Rat
Specificity	Detects endogenous human, mouse and rat ATG3 in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human ATG3 Met1-His287 Accession # Q9NT62
Formulation	Lyophilized from a 0.2 µm 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

<p>Western Blot</p>  <p>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.</p>	<p>Immunocytochemistry</p>  <p>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.</p>
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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. <ul style="list-style-type: none"> ● 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.