

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat ATG5 in Western blots.
Source	Monoclonal Mouse IgG _{2B} Clone # 603813
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human ATG5 Asn99-Thr193 Accession # Q9H1Y0
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	0.5 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	Immersion fixed paraffin-embedded human small intestine
Simple Western	25 µg/mL	See Below

DATA

Western Blot

Detection of Human, Mouse, and Rat ATG5 by Western Blot.
Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, CH-1 mouse B cell lymphoma cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF Membrane was probed with 0.5 µg/mL of Mouse Anti-Human/Mouse/Rat ATG5 Monoclonal Antibody (Catalog # MAB5294) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for ATG5 at approximately 50 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Simple Western

Detection of Human and Rat ATG5 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and PC-12 rat adrenal pheochromocytoma cell line, loaded at 0.2 mg/mL. A specific band was detected for ATG5 at approximately 51 kDa (as indicated) using 25 µg/mL of Mouse Anti-Human/Mouse/Rat ATG5 Monoclonal Antibody (Catalog # MAB5294). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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

ATG5 (Autophagy-related Protein), also known as APG5L and Apoptosis-specific Protein, is a ubiquitous 32 kDa member of the ATG family of proteins. ATG5 exists as a covalent heterodimer with ATG12 through the creation of a Lys-Gly linkage. The ATG5:ATG12 heterodimer associates noncovalently with an ATG16 multimer to generate autophagosomes. Human ATG5 is 275 amino acids in length and contains N- and C-terminal ubiquitin-like domains (aa 15-105 and 187-275) separated by a helix-rich linker region that contains a dimerizing Lys at position 130. There are two potential alternate start sites at Met80 and Met173. Over aa 99-193, human ATG5 is 97% aa identical to mouse ATG5.