Human GABARAP Antibody

Monoclonal Rat IgG_{2A} Clone # 853641

Catalog Number: MAB8574

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human GABARAP in direct ELISAs.
Source	Monoclonal Rat IgG_{2A} Clone # 853641
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human GABARAP Accession # O95166
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 either lyophilized or as a 0.2 µm filtered solution in PBS.

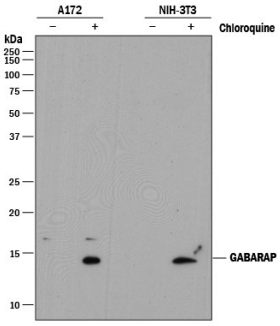
APPLICATIONS

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	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

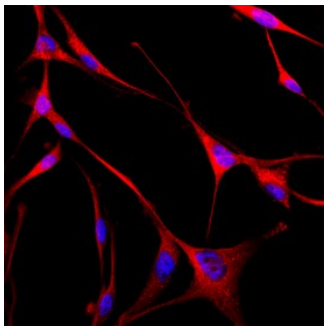
DATA

Western Blot



Detection of Human and Mouse GABARAP by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line and NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 50 µM Chloroquine for 20 hours. PVDF membrane was probed with 2 µg/mL of Rat Anti-Human GABARAP Monoclonal Antibody (Catalog # MAB8574) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). Specific bands were detected for GABARAP at approximately 14 & 16 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 7.

Immunocytochemistry



GABARAP in A172 Human Cell Line. GABARAP was detected in immersion fixed A172 human glioblastoma cell line using Rat Anti-Human GABARAP Monoclonal Antibody (Catalog # MAB8574) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to Golgi apparatus and cytoplasmic vesicles. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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

γ-Aminobutyric Acid Receptor-associated Protein (GABARAP), also known as Apg8p1, is 117 amino acid (aa) protein with a predicted molecular weight of 14 kDa. It is a member of the GABARAP subfamily of the Autophagy-related 8 (Atg8) family of proteins (1). The GABARAP subfamily also includes GABARAPL1. GABARAP/Apg8p1 has 100% aa sequence identity with its mouse and rat orthologs and is orthologous to yeast Atg8. Atg8 family members show structural similarity with Ubiquitin, but lack aa sequence similarity. GABARAP/Apg8p1 was first described for its putative involvement GABA A Receptor trafficking (2,3). However, it is best known for its role in autophagy (1,4). GABARAP/Apg8p1 covalently attaches to phosphatidylethanolamine (PE) in the phagophore (autophagosome precursor) membrane using a Ubiquitin-like conjugation system that includes Ubiquitin-activating (E1)-, Ubiquitin-conjugating (E2)-, and Ubiquitin Ligase (E3)-like enzymes. Here it is involved in the later stages of autophagosome maturation (4,5). It may also be involved in cargo recruitment to autophagosomes (1).

References:

1. Shpilka, T. *et al.* (2011) Genome Biol. **12**:226.
2. Wang, H. *et al.* (1999) Nature **397**:69.
3. Leil, T.A. *et al.* (2004) J. Neurosci. **24**:11429.
4. Weidberg, H. *et al.* (2010) EMBO J. **29**:1792.
5. Weidberg, H. *et al.* (2011) Ann. Rev. Biochem. **80**:125.

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