

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse PRAS40 when phosphorylated at T246
Source	Monoclonal Mouse IgG _{2A} Clone # 760502
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human PRAS40 T246 site
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
Immunocytochemistry	8-25 µg/mL	See Below

DATA

Western Blot

Detection of Human and Mouse Phospho-PRAS40 (T246) by Western Blot. Western blot shows lysates of MCF-7 human breast cancer cell line and NIH-3T3 mouse embryonic fibroblast cell line untreated (-) or treated (+) with 100 ng/mL Recombinant Human IGF-I (Catalog # 291-G1) for 20 minutes and 10 ng/mL Recombinant Human PDGF-BB (Catalog # 220-BB) for 5 minutes. PVDF membrane was probed with 0.5 µg/mL of Mouse Anti-Human Phospho-PRAS40 (T246) Monoclonal Antibody (Catalog # MAB6890) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Phospho-PRAS40 (T246) at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry

Phospho-PRAS40 (T246) in MCF-7 Human Cell Line. PRAS40 phosphorylated at T246 was detected in immersion fixed MCF-7 human breast cancer cell line using Mouse Anti-Human/Mouse Phospho-PRAS40 (T246) Monoclonal Antibody (Catalog # MAB6890) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

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

PRAS40 (Proline-rich AKT1 substrate 1; also Akt1S1 and p39) is a 40-42 kDa cytoplasmic phosphoprotein that lacks generally recognized structural motifs. It is widely expressed and is considered to be key regulator of mTORC1 (mTOR plus raptor and GβL), a complex through which Akt signals into the cell. Through phosphorylation, mTORC1 activity is upregulated by PRAS40. In particular, nonphosphorylated PRAS40 binds to and serves as a negative regulator of mTORC1 activity. Upon Insulin signaling, PRAS40 is phosphorylated on Thr246, Ser221 and Ser183. This causes it to bind 14-3-3 and results in its dissociation from mTORC1, freeing up mTOR to regulate (positively or negatively) protein synthesis. Human PRAS40 is 256 amino acids (aa) in length. It contains one extended Pro-rich region (aa 35-96) plus at least nine utilized Ser/Thr phosphorylation sites. There is one alternative start site at Met131. Over aa 119-256, human PRAS40 shares 93% aa identity with mouse PRAS40.