

Anti-ECT2 pT790 (RABBIT) Antibody

ECT2 phospho T790 Antibody Catalog # ASR5356

Specification

Anti-ECT2 pT790 (RABBIT) Antibody - Product Information

Host Conjugate Target Species Reactivity Clonality Application Application Note Rabbit

Unconjugated

Human Human Polyclonal WB, E, IP, I, LCI

This affinity-purified antibody has been tested for use in ELISA, western blot and immunoprecipitation. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 101 kDa in size by western blotting in the appropriate cell lysate or extract. Less than 2% reactivity is observed against the

non-phosphorylated form of the

immunizing peptide. This antibody is believed to be phospho specific for ECT2 phosphorylated at the pT790 residue. Experiments to generate more conclusive evidence are currently under preparation.

Liquid (sterile filtered)

0.02 M Potassium Phosphate, 0.15 M

Sodium Chloride, pH 7.2

This affinity purified antibody was prepared from whole rabbit serum

produced by repeated immunizations with a synthetic peptide corresponding to an internal region of human ECT2 protein.

0.01% (w/v) Sodium Azide

Physical State Buffer

Immunogen

Preservative

Anti-ECT2 pT790 (RABBIT) Antibody - Additional Information

Gene ID 1894

Other Names 1894

Purity

This affinity-purified antibody is directed against the phosphorylated form of human ECT2 protein at the pT790 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human ECT2 protein at the pT790 residue. By ELISA the antibody is specific for the phosphorylated form of the





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protein. Reactivity with non-phosphorylated human ECT2 is minimal by ELISA, but western blot shows specific staining of interphase cell lysates, which are predicted to contain non-phosphorylated ECT2. Additional experiments are underway to clarify antibody specificity. A BLAST analysis was used to suggest cross reactivity with ECT2 protein from human, mouse, rat, zebrafish, chimpanzee, chicken, and dog based on 100% homology with the immunizing sequence. Reactivity against homologues from other sources is not known.

Storage Condition

Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

Precautions Note

This product is for research use only and is not intended for therapeutic or diagnostic applications.

Anti-ECT2 pT790 (RABBIT) Antibody - Protein Information

Name ECT2 (HGNC:3155)

Function

Guanine nucleotide exchange factor (GEF) that catalyzes the exchange of GDP for GTP. Promotes guanine nucleotide exchange on the Rho family members of small GTPases, like RHOA, RHOC, RAC1 and CDC42. Required for signal transduction pathways involved in the regulation of cytokinesis. Component of the centralspindlin complex that serves as a microtubule-dependent and Rho-mediated signaling required for the myosin contractile ring formation during the cell cycle cytokinesis. Regulates the translocation of RHOA from the central spindle to the equatorial region. Plays a role in the control of mitotic spindle assembly; regulates the activation of CDC42 in metaphase for the process of spindle fibers attachment to kinetochores before chromosome congression. Involved in the regulation of epithelial cell polarity; participates in the formation of epithelial tight junctions in a polarity complex PARD3-PARD6-protein kinase PRKCQ-dependent manner. Plays a role in the regulation of neurite outgrowth. Inhibits phenobarbital (PB)-induced NR1I3 nuclear translocation. Stimulates the activity of RAC1 through its association with the oncogenic PARD6A- PRKCI complex in cancer cells, thereby acting to coordinately drive tumor cell proliferation and invasion. Also stimulates genotoxic stress-induced RHOB activity in breast cancer cells leading to their cell death.

Cellular Location

Nucleus. Cytoplasm. Cytoplasm, cytoskeleton, spindle. Cleavage furrow. Midbody. Cell junction. Cell junction, tight junction. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Note=Seguestered within the nucleus during interphase (PubMed:10579713). Dispersed throughout the cytoplasm upon breakdown of the nuclear envelope during mitosis (PubMed:10579713). Colocalizes with the central spindlin complex to the mitotic spindles during anaphase/metaphase, the cleavage furrow during telophase and at the midbody at the end of cytokinesis (PubMed:10579713). Colocalized with RhoA at the midbody (PubMed:10579713). Its subcellular localization to tight junction is increased by calcium (PubMed:15254234).

Tissue Location

Expressed in lung epithelial cells (at protein level). Expressed in squamous cell carcinoma, primary non-small cell lung cancer tumors and lung adenocarcinoma

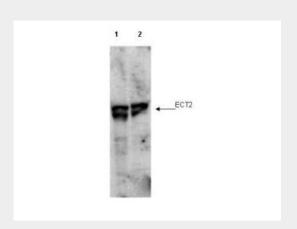
Anti-ECT2 pT790 (RABBIT) Antibody - Protocols



Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- <u>Immunohistochemistry</u>
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-ECT2 pT790 (RABBIT) Antibody - Images



Western blot using Rockland's affinity purified anti-ECT2 pT790 antibody shows detection of endogenous phosphorylated ECT2 (arrowhead) present in cell lysates from interphase (lane 1) and mitotic (lane 2) HeLa cells. Despite specific staining of interphase cells, this reagent is believed to be phospho specific based on ELISA results using both phosphorylated and non-phosphorylated immunizing peptide. After SDS-PAGE and transfer, the membrane was probed with the primary antibody diluted to 1:1,000. Personal Communication, Toru Miki, CCR-NCI, Bethesda, MD.

Anti-ECT2 pT790 (RABBIT) Antibody - Background

This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. ECT2, also known as epithelial cell transforming sequence 2 oncogene, was originally isolated as a transforming gene from epithelial cells. ECT2 catalyzes guanine nucleotide exchange on the small GTPases, RhoA, Rac1, and Cdc42. ECT2 may be phosphorylated during G2 and M phases, and phosphorylation may be required for its exchange activity. Unlike other known guanine nucleotide exchange factors for Rho GTPases, ECT2 exhibits nuclear localization in interphase, spreads throughout the cytoplasm in prometaphase, and is condensed in the midbody during cytokinesis. Expression of dominant-negative ECT2 or microinjection of affinity-purified anti-ECT2 antibody into interphase cells strongly inhibits cytokinesis. These results suggest that ECT2 is an important link between the cell cycle machinery and Rho signaling pathways involved in the regulation of cell division. Phosphorylation at T790 or S375 significantly affects the catalytic activity of ECT2.