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Anti-Ran Antibody Picoband™ (monoclonal, 5D5)

Catalog # ABO14880

Product Information

Application	WB, IF, ICC, FC
Primary Accession	P62826
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Ran Antibody Picoband™ (monoclonal, 5D5) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	5901
Other Names	GTP-binding nuclear protein Ran, 3.6.5.-, Androgen receptor-associated protein 24, GTPase Ran, Ras-like protein TC4, Ras-related nuclear protein, RAN, ARA24 {ECO:0000303|PubMed:10400640}
Calculated MW	24423
Application Details	Western blot, 0.1-0.5 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cytosol. Nucleus. Nucleus envelope. Cytoplasm. Melanosome.
Tissue Specificity	Expressed in a variety of tissues.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 5D5
Immunogen	E. coli-derived human Ran recombinant protein (Position: A2-L216). Human Ran shares 100% amino acid (aa) sequence identity with both mouse and rat Ran.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six

months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	RAN
Synonyms	ARA24 {ECO:0000303|PubMed:10400640}
Function	GTPase involved in nucleocytoplasmic transport, participating both to the import and the export from the nucleus of proteins and RNAs (PubMed:10400640, PubMed:17209048, PubMed:26272610, PubMed:27306458, PubMed:8276887, PubMed:8636225, PubMed:8692944, PubMed:8896452, PubMed:9351834, PubMed:9428644, PubMed:9822603). Switches between a cytoplasmic GDP- and a nuclear GTP-bound state by nucleotide exchange and GTP hydrolysis (PubMed:11336674, PubMed:26272610, PubMed:29040603, PubMed:7819259, PubMed:8636225, PubMed:8692944, PubMed:8896452, PubMed:9351834, PubMed:9428644, PubMed:9822603). Nuclear import receptors such as importin beta bind their substrates only in the absence of GTP-bound RAN and release them upon direct interaction with GTP-bound RAN, while export receptors behave in the opposite way. Thereby, RAN controls cargo loading and release by transport receptors in the proper compartment and ensures the directionality of the transport (PubMed:8896452, PubMed:9351834, PubMed:9428644). Interaction with RANBP1 induces a conformation change in the complex formed by XPO1 and RAN that triggers the release of the nuclear export signal of cargo proteins (PubMed:20485264). RAN (GTP-bound form) triggers microtubule assembly at mitotic chromosomes and is required for normal mitotic spindle assembly and chromosome segregation (PubMed:10408446, PubMed:29040603). Required for normal progress through mitosis (PubMed:12194828, PubMed:29040603, PubMed:8421051). The complex with BIRC5/survivin plays a role in mitotic spindle formation by serving as a physical scaffold to help deliver the RAN effector molecule TPX2 to microtubules (PubMed:18591255). Acts as a negative regulator of the kinase activity of VRK1 and VRK2 (PubMed:18617507). Enhances AR- mediated transactivation. Transactivation decreases as the poly-Gln length within AR increases (PubMed:10400640).
Cellular Location	Nucleus. Nucleus envelope. Cytoplasm, cytosol Cytoplasm. Melanosome Note=Predominantly nuclear during interphase (PubMed:10679025, PubMed:12194828, PubMed:8421051). Becomes dispersed throughout the cytoplasm during mitosis (PubMed:12194828, PubMed:8421051). Identified by mass spectrometry in melanosome fractions from stage I to stage IV (PubMed:17081065).
Tissue Location	Expressed in a variety of tissues.

Background

RAN (ras-related nuclear protein) is a small GTP binding protein belonging to the RAS superfamily that is essential for the translocation of RNA and proteins through the nuclear pore complex. The RAN protein is also involved in control of DNA synthesis and cell cycle progression. Nuclear localization of RAN requires the presence of regulator of chromosome condensation 1 (RCC1). Mutations in RAN disrupt DNA synthesis. Because of its many functions, it is likely that RAN interacts with several other proteins. RAN regulates formation and organization of the microtubule network independently of its role in the nucleus-cytosol exchange of macromolecules. RAN could be a key signaling molecule regulating microtubule polymerization during mitosis. RCC1 generates a high local concentration of RAN-GTP around chromatin which, in turn, induces the local nucleation of microtubules. RAN is an androgen receptor (AR) coactivator that binds differentially with different lengths of polyglutamine within the androgen receptor. Polyglutamine repeat

expansion in the AR is linked to Kennedy's disease (X-linked spinal and bulbar muscular atrophy). RAN coactivation of the AR diminishes with polyglutamine expansion within the AR, and this weak coactivation may lead to partial androgen insensitivity during the development of Kennedy's disease.

Images

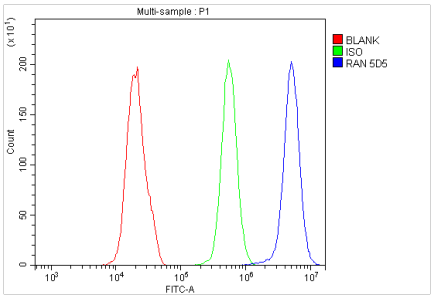


Figure 3. Flow Cytometry analysis of PC-3 cells using anti-Ran antibody (M00204-1).
Overlay histogram showing PC-3 cells stained with M00204-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ran Antibody (M00204-1,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

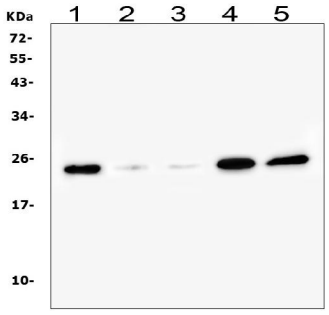


Figure 2. Western blot analysis of Ran using anti-Ran antibody (M00204-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat testis tissue lysates,
Lane 2: mouse lung tissue lysates,
Lane 3: mouse kidney tissue lysates,
Lane 4: mouse testis tissue lysates,
Lane 5: mouse Neuro-2a whole cell lysates,
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Ran antigen affinity purified monoclonal antibody (Catalog # M00204-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Ran at approximately 24KD. The expected band size for Ran is at 24KD.

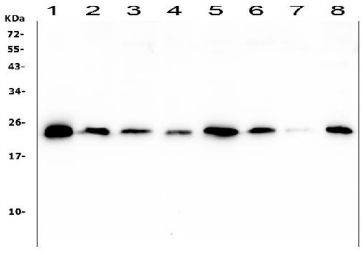


Figure 1. Western blot analysis of Ran using anti-Ran antibody (M00204-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HL-60 whole cell lysates,
Lane 2: human T-47D whole cell lysates,
Lane 3: human A549 whole cell lysates,
Lane 4: human U2OS whole cell lysates,
Lane 5: human THP-1 whole cell lysates,
Lane 6: human HepG2 whole cell lysates,
Lane 7: human PANC-1 whole cell lysates,

Lane 8: human SW620 whole cell lysates,
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Ran antigen affinity purified monoclonal antibody (Catalog # M00204-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for Ran at approximately 24KD. The expected band size for Ran is at 24KD.

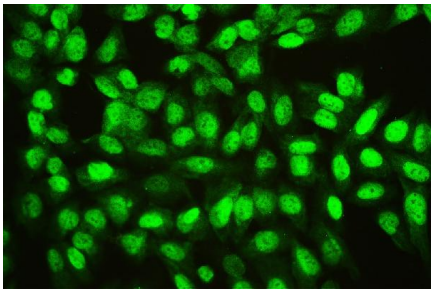


Figure 4. IF analysis of Ran using anti-Ran antibody (M00204-1).
Ran was detected in immunocytochemical section of U20S cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-Ran Antibody (M00204-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.