10320 Camino Santa Fe, Suite G
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Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13)

Catalog # ABO14974

Specification

Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	Q14008
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Additional Information

Gene ID 9793

Other Names
Cytoskeleton-associated protein 5, Colonic and hepatic tumor overexpressed gene protein, Ch-TOG, CKAP5, KIAA0097

Calculated MW
225 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human, Rat
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen
E.coli-derived human ch TOG/CKAP5 recombinant protein (Position: M1-L221).

Purification
Immunogen affinity purified.

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.**

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Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Protein Information

Name CKAP5

Synonyms KIAA0097

Function
Binds to the plus end of microtubules and regulates microtubule dynamics and microtubule organization. Acts as a processive microtubule polymerase. Promotes cytoplasmic microtubule nucleation and elongation. Plays a major role in organizing spindle poles. In spindle formation protects kinetochore microtubules from depolymerization by KIF2C and has an essential role in centrosomal microtubule assembly independently of KIF2C activity. Contributes to centrosome integrity. Acts as a component of the TACC3/ch-TOG/clathrin complex proposed to contribute to stabilization of kinetochore fibers of the mitotic spindle by acting as inter-microtubule bridge. The TACC3/ch- TOG/clathrin complex is required for the maintenance of kinetochore fiber tension (PubMed:23532825). Enhances the strength of NDC80 complex-mediated kinetochore-tip microtubule attachments (PubMed:27156448).

Cellular Location
Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Cytoplasm, cytoskeleton, spindle pole. Cytoplasm, cytoskeleton, spindle. Chromosome, centromere, kinetochore. Note=Detected on centrosomes and kinetochores during interphase and mitosis independently from TACC3 and clathrin. Located to spindle poles and microtubules during mitosis. In complex with TACC3 localized to microtubule plus-ends in mitosis and interphase. In complex with TACC3 and clathrin localized to inter- microtubule bridges in mitotic spindles. Accumulation sites at microtubule plus ends protruded approximately 100 nm from MAPRE1/EB1 sites in interphase cells.

Tissue Location
Overexpressed in hepatomas and colonic tumors. Also expressed in skeletal muscle, brain, heart, placenta, lung, liver, kidney and pancreas. Expression is elevated in the brain; highly expressed in the Purkinje cell bodies of the cerebellum

Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Protocols

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

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Images

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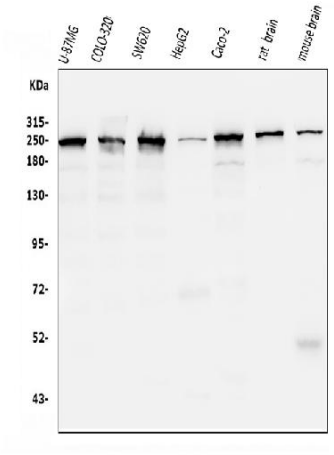

Figure 1. Western blot analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human U-87MG whole cell lysates,
Lane 2: human COLO-320 whole cell lysates,
Lane 3: human SW620 whole cell lysates,
Lane 4: human HEPG2 whole cell lysates,
Lane 5: human CACO-2 whole cell lysates,
Lane 6: rat brain tissue lysates,
Lane 7: mouse brain tissue 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-Ch TOG/CKAP5 antigen affinity purified monoclonal antibody (Catalog # M05324) 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 Ch TOG/CKAP5 at approximately 225KD. The expected band size for Ch TOG/CKAP5 is at 225KD.

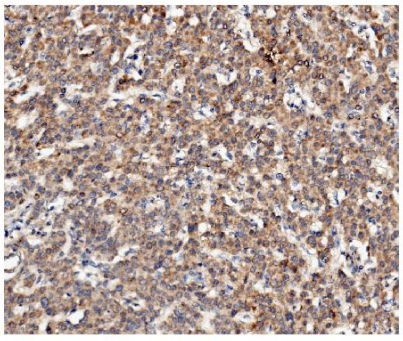

Figure 2. IHC analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Ch TOG/CKAP5 was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ch TOG/CKAP5 Antibody (M05324) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as

the chromogen.

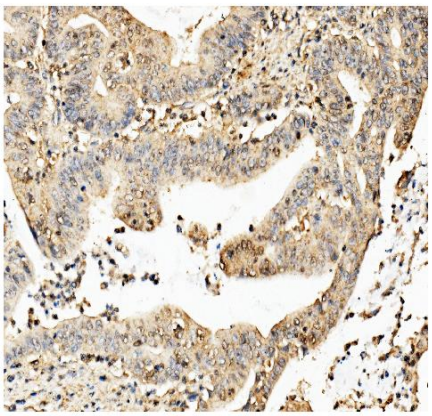

Figure 3. IHC analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Ch TOG/CKAP5 was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ch TOG/CKAP5 Antibody (M05324) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

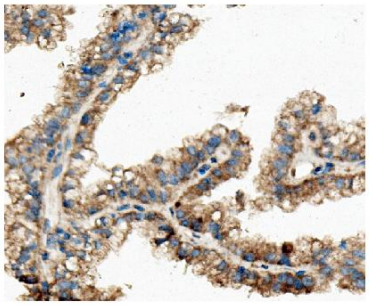

Figure 4. IHC analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Ch TOG/CKAP5 was detected in paraffin-embedded section of human renal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ch TOG/CKAP5 Antibody (M05324) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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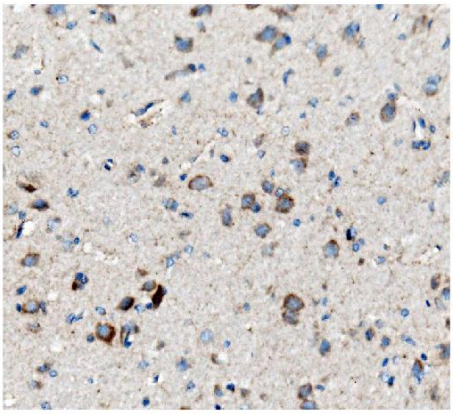

Figure 5. IHC analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Ch TOG/CKAP5 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Ch TOG/CKAP5 Antibody (M05324) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

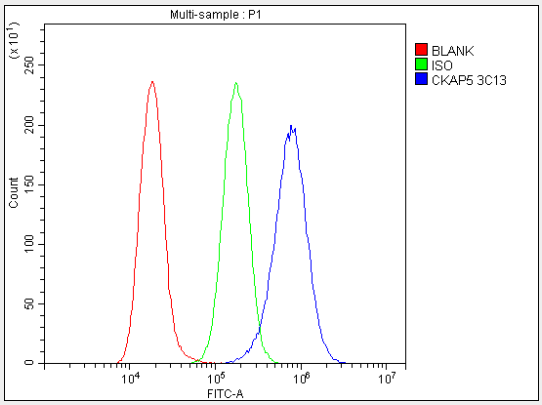

Figure 6. Flow Cytometry analysis of A549 cells using anti-Ch TOG/CKAP5 antibody (M05324).
Overlay histogram showing A549 cells stained with M05324 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Ch TOG/CKAP5 Antibody (M05324, 1 µg/1x10^6 cells) for 30 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.

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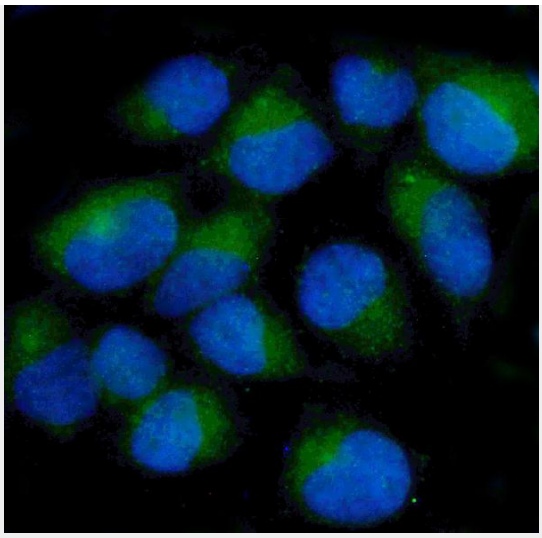

Figure 7. IF analysis of Ch TOG/CKAP5 using anti-Ch TOG/CKAP5 antibody (M05324).
Ch TOG/CKAP5 was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Ch TOG/CKAP5 Antibody (M05324) 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. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Anti-ch TOG/CKAP5 Antibody Picoband™ (monoclonal, 3C13) - Background

Cytoskeleton-associated protein 5 is a microtubule-associated protein that in humans is encoded by the CKAP5 gene. It is mapped to 11p11.2. This gene encodes a cytoskeleton-associated protein which belongs to the TOG/XMAP215 family. The N-terminal half of this protein contains a microtubule-binding domain and the C-terminal half contains a KXGS motif for binding tubulin dimers. This protein has two distinct roles in spindle formation; it protects kinetochore microtubules from depolymerization and plays an essential role in centrosomal microtubule assembly. This protein may be necessary for the proper interaction of microtubules with the cell cortex for directional cell movement. It also plays a role in translation of the myelin basic protein (MBP) mRNA by interact ernatively spliced transcript variants encoding different isoforms have been identified.