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Polyclonal Antibody to Inscuteable - Purified

Alternate names: Protein inscuteable homolog

Catalog No.: AP56115PU-N

Quantity: 0.1 mg

Concentration: lot-specific

Background: Inscuteable (minsc or protein inscuteable homolog) is known to act as key regulator of spindle orientation in Drosophila. During mammalian neurogenesis, it is expressed temporally and spatially and is thought to influence the orientation of neural progenitor divisions, which are important for cell differentiation in the retina. Aside from its ability to influence the cytoskeletal structure of neural progenitor cells, it is suggested that Gi-alpha and inscuteable may play a role in regulating cellular stress associated with protein-processing pathologies. In particular, inscuteable augments the distribution of AGS3 within the aggresome whereas the AGS3 binding partner, Gi-alpha, is shown to rescue it from the aggresome.

Uniprot ID: Q1MX18

NCBI: NP_001027024.3

GeneID: 387755

Host: Rabbit

Immunogen: KLH-conjugated linear peptide corresponding to the human protein inscuteable homolog.

Format: **State:** Liquid purified Ig fraction
Purification: Protein A Chromatography
Buffer System: 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide.

Applications: **Immunohistochemistry**: 2 µg/ml from a representative lot detected Inscuteable in paraffin-embedded human kidney and colon tissues.
Western Blot: A previous lot of this antibody was used to detect Inscuteable in L6 cell lysates.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Molecular Weight: ~63 kDa observed. Uncharacterized bands may be observed at ~70-85 kDa and ~225 kDA in some cell lysates.

Specificity: **Species:** Rat, Human.
Other species not tested.

Storage: Store undiluted at 2-8°C.
Shelf life: one year from despatch.

General Readings: 1. Zigman M, Cayouette M, Charalambous C, Schleiffer A, Hoeller O, Dunican D, et al. Mammalian inscuteable regulates spindle orientation and cell fate in the developing

retina. Neuron. 2005 Nov 23;48(4):539-45. PubMed PMID: 16301171.
2. Izaki T, Kamakura S, Kohjima M, Sumimoto H. Two forms of human Inscuteable-related protein that links Par3 to the Pins homologues LGN and AGS3. Biochem Biophys Res Commun. 2006 Mar 24;341(4):1001-6. Epub 2006 Jan 24. PubMed PMID: 16458856.
3. Vural A, Oner S, An N, Simon V, Ma D, Blumer JB, et al. Distribution of activator of G-protein signaling 3 within the aggresomal pathway: role of specific residues in the tetratricopeptide repeat domain and differential regulation by the AGS3 binding partners Gi(alpha) and mammalian inscuteable. Mol Cell Biol. 2010 Mar;30(6):1528-40. doi: 10.1128/MCB.01018-09. Epub 2010 Jan 11. PubMed PMID: 20065032.

Protocols:

Western Blotting

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA or milk with 0.05% Tween®-20 surfactant for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of the primary antibody, diluted in freshly prepared 5% BSA or milk for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Visualize with enhanced chemiluminescence (ECL) method of choice.

Pictures:

Immunohistochemistry Analysis:
Representative lot data.
Parrafin-embedded human kidney (Fig. 1) and colon (Fig. 2) tissues were prepared using heat-induced epitope retrieval in citrate buffer, pH 6.0. Immunostaining was performed using 2 µg/mL of Cat. No. AP56115PU-N, Anti-Inscuteable.
Reactivity was detected using the IHC-Select® Detection Kit. Positive staining was observed in human kidney and liver tissues.

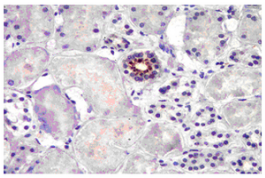
Fig. 1

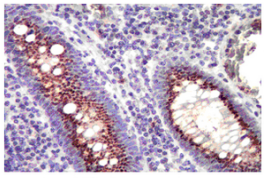
Fig. 2

Western Blotting Analysis:
Representative lot data.
Human liver tissue lysate was probed with Anti-Inscuteable (1 µg/mL).
Proteins were visualized using a Donkey Anti-Rabbit IgG secondary antibody conjugated to HRP and a chemiluminescence detection system.
Arrow indicates Inscuteable (~63 kDa).
Uncharacterized bands may be observed at ~70-85 kDa and ~225 kDA in some cell lysates.