

NECAP 1 (G-14): sc-161932

BACKGROUND

Clathrin-coated vesicles (CCVs) select cargo for endocytotic entry into cells and generate carrier vesicles for transport between the endosomal system and *trans*-Golgi network (TGN). NECAP 1 and NECAP 2 are essential protein paralogues for clathrin-mediated membrane trafficking that are enriched in CCV coats. NECAP 1 (NECAP endocytosis associated 1), also known as adap-tin ear-binding coat-associated protein 1, is a 275 amino acid cell membrane protein belonging to the NECAP family that localizes to the clathrin-coated vesicle membrane. NECAP 1 is encoded by a gene that maps to human chromosome 12p13.31 and contains two WXXF motifs which are used to mediate binding of accessory proteins to ear domains of various proteins such as AP-1, AP-2 and GGAs. Expressed primarily in brain and existing as two alternatively spliced isoforms, NECAP 1 colocalizes with AP-2 at the plasma membrane by binding AP-2s α -ear domain.

REFERENCES

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2. Ritter, B., Philie, J., Girard, M., Tung, E.C., Blondeau, F. and McPherson, P.S. 2003. Identification of a family of endocytic proteins that define a new α -Adaptin ear-binding motif. *EMBO Rep.* 4: 1089-1095.
3. Ritter, B., Blondeau, F., Denisov, A.Y., Gehring, K. and McPherson, P.S. 2004. Molecular mechanisms in clathrin-mediated membrane budding revealed through subcellular proteomics. *Biochem. Soc. Trans.* 32: 769-773.
4. Mattera, R., Ritter, B., Sidhu, S.S., McPherson, P.S. and Bonifacino, J.S. 2004. Definition of the consensus motif recognized by γ -adaptin ear domains. *J. Biol. Chem.* 279: 8018-8028.
5. McPherson, P.S. and Ritter, B. 2005. Peptide motifs: building the clathrin machinery. *Mol. Neurobiol.* 32: 73-87.

CHROMOSOMAL LOCATION

Genetic locus: NECAP1 (human) mapping to 12p13.31; Necap1 (mouse) mapping to 6 F2.

SOURCE

NECAP 1 (G-14) is an affinity purified goat polyclonal antibody raised against a peptide mapping within an internal region of NECAP 1 of human origin.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-161932 P, (100 μ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

NECAP 1 (G-14) is recommended for detection of NECAP 1 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000); non cross-reactive with NECAP 2.

NECAP 1 (G-14) is also recommended for detection of NECAP 1 in additional species, including equine.

Suitable for use as control antibody for NECAP 1 siRNA (h): sc-95850, NECAP 1 siRNA (m): sc-149896, NECAP 1 shRNA Plasmid (h): sc-95850-SH, NECAP 1 shRNA Plasmid (m): sc-149896-SH, NECAP 1 shRNA (h) Lentiviral Particles: sc-95850-V and NECAP 1 shRNA (m) Lentiviral Particles: sc-149896-V.

Molecular Weight of NECAP 1: 40 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, mouse brain extract: sc-2253 or mouse cerebellum extract: sc-2403.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.