Product Datasheet

E6AP/UBE3A Antibody - BSA Free
NB500-239

Unit Size: 100 ul

Store at 4C. Do not freeze.

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Publications: 1

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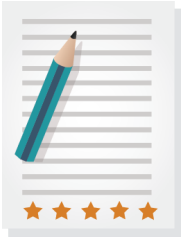
Updated 2/21/2025 v.20.1



NB500-239

E6AP/UBE3A Antibody - BSA Free

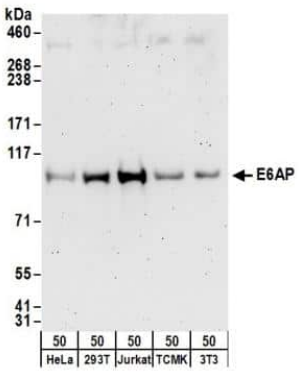
Product Information	
Unit Size	100 ul
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)

Product Description	
Host	Rabbit
Gene ID	7337
Gene Symbol	UBE3A
Species	Human, Mouse
Reactivity Notes	Based on 100% sequence identity, this antibody is predicted to react with Panda, Orangutan, Rhesus Monkey, Gorilla, Chimpanzee, White-tufted-ear marmoset, Crabeating macaque, Naked mole rat, Thirteen-lined ground squirrel and Northern white-cheeked gibbon.
Immunogen	A synthetic peptide made to a portion of human ubiquitin protein ligase E3A encoded within exon 6 (LocusLink ID 7337).

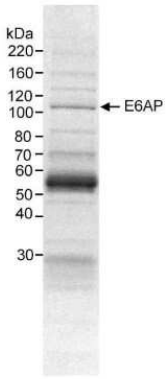
Product Application Details	
Applications	Western Blot, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000 - 1:5000, Immunoprecipitation 1 - 4 ug/mg of lysate
Application Notes	It recognizes a band at ~104 kDa in Western blot, representing E6AP (in cytosolic or nuclear extracts). *The investigator should determine the optimal working dilution for a specific application.

Images

Western Blot: E6AP/UBE3A Antibody [NB500-239] - Detection of Human and Mouse E6AP by Western Blot. Samples: Whole cell lysate (50 ug) from HeLa, 293T, Jurkat, mouse TCMK-1, and mouse NIH3T3 cells. Antibodies: Affinity purified rabbit anti-E6AP antibody NB500-239 used for WB at 0.4 ug/ml. Detection: Chemiluminescence with an exposure time of 3 minutes.



Western Blot: E6AP/UBE3A Antibody [NB500-239] - Cytoplasmic (S100) fraction (10 mg) from HeLa cells. Antibody used at 20 ug/10 mg extract.



Publications

Sato M, Stryker MP. Genomic imprinting of experience-dependent cortical plasticity by the ubiquitin ligase gene Ube3a. Proc Natl Acad Sci U S A 2010-03-01 [PMID: 20212164]

Procedures

Protocol specific for UBE3A Antibody (NB500-239)
Nuclear Extract and Cytoplasmic Fraction Preparation protocol for UBE3A Antibody (NB500-239):
Nuclear Extract and Cytoplasmic Fraction Preparation

1. Nuclear extracts (NE) and cytoplasmic fractions (S100) were prepared by Dignam's method (Dignam, Lebovitz, and Roeder, Nucleic Acids Res. 11: 1475-1489. 1983).
2. 100 liters of HeLa cell culture were harvested and washed 3 times with cold PBS.
3. The packed-cell volume (PCV) was measured, and the cell pellet was gently resuspended with 5 PCVs of hypotonic buffer (10 mM HEPES-KOH [pH 8], 10 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 0.2 mM PMSF).
4. Cells were incubated on ice for 10 minutes and then pelleted by centrifugation at 1,800xg for 10 minutes.
5. Hypotonic buffer was added to 2 PCVs, and cells were resuspended and then homogenized with 15 strokes using a pestle B in a Dounce glass homogenizer until the cells were more than 90% lysed, as determined by a light microscope.
6. The lysate was centrifuged at 20,000xg for 30 minutes at 4 degrees Celcius.
7. The supernatant was saved for S100 fraction, and the pellet was saved to measure the packed nuclear volume (PNV).
8. 0.4 ml of extraction buffer (20 mM HEPES-KOH [pH 8], 0.6 M KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 25% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) per ml of PNV was added.
9. Cell nuclei were homogenized with 10 strokes of pestle A in the homogenizer.
10. Suspension was stirred at 4 degrees Celcius for 30 minutes and centrifuged for 30 minutes at 20,000xg.
11. The supernatant (nuclear extract) was aliquotted for use.
12. The S100 fraction (resulting supernatant) was mixed with 0.11 volume of high-salt buffer (20 mM HEPES-KOH [pH 8], 1.2 M KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) and centrifuged at 100,000xg for 60 minutes at 4 degrees Celcius.
13. This supernatant was dialyzed for 2 hours at 4 degrees Celcius.
14. The sample was centrifuged for 30 minutes at 20,000xg and the supernatant (S100) was aliquotted for use.

Immunoprecipitation

Antibody characterization:

1. HeLa NE and S100 were diluted with 1 volume of RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris [pH 8]).
2. Cleared by spinning at 100,000 g for 20 minutes at 4 degrees Celcius.
3. 1 ml of supernatant (~10 mg total protein) was mixed with 20 ug of primary antibody (NB 500-239) and rotated overnight at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 2 hours at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the 10% RIPA in PBS.
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

Complex purification:

1. NE and S100 were cleared by spinning at 20,000 g for 30 minutes at 4 degrees Celcius.
2. 1.5 ml of supernatant (~15 mg total protein) was mixed with 20 ug of primary antibody (NB 500-239) and rotated for 4 hours at 4 degrees Celcius.
3. Sample and antibody mixture were centrifuged at 15,000 g for 20 minutes at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 1 hour at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the NETN buffer (20 mM Tris-HCl [pH 8], 100 mM NaCl, 1 mM EDTA, 0.5% NP-40).
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

*If an insufficient amount of protein is purified for identification from 15 mg of extract, carry out the same procedure using 50-100 mg of extract to increase the amount of purified protein yield.

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Products Related to NB500-239

NB800-PC1	HeLa Whole Cell Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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