

# E6-AP (EE-7): sc-100614

## BACKGROUND

E6-associating protein (E6-AP), also designated UBE4, is a component of the ubiquitin-mediated proteolytic pathway, which selectively targets proteins for degradation by the 26S proteasome. Ubiquitin (Ub) is directly conjugated to protein substrates by the transfer of Ub from an E2 ubiquitin conjugating enzyme to the target protein. This conjugation is facilitated by the enzymatic activity of E3 ubiquitin ligase family members such as E6-AP. Several substrates of E6-AP have been identified and include the tumor suppressor protein p53 and the mammalian homolog of Rad23, HHR23A. Previous studies have indicated that E6-AP associates with the human papillomavirus E6 oncoprotein, which complexes with p53 and thereby potentiates E6-AP mediated ubiquitination of p53. Genetic mutations that impair E6-AP activity result in the accumulation of p53 in the cytoplasm, and, in many instances, these mutations are associated with the development of the rare neurodevelopmental disorder Angelman syndrome (AS), which is characterized by severe motor dysfunction and mental retardation.

## REFERENCES

- Jentsch, S. 1992. The ubiquitin-conjugation system. *Annu. Rev. Genet.* 26: 179-207.
- Huibregtse, J.M., et al. 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Mol. Cell. Biol.* 13: 775-784.
- Haas, A.L., et al. 1997. Pathways of ubiquitin conjugation. *FASEB J.* 11: 1257-1268.
- Yamamoto, Y., et al. 1997. The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. *Genomics* 41: 263-266.
- Malzac, P., et al. 1998. Mutation analysis of UBE3A in Angelman syndrome patients. *Am. J. Hum. Genet.* 62: 1353-1360.
- Nawaz, Z., et al. 1999. The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. *Mol. Cell. Biol.* 19: 1182-1189.

## CHROMOSOMAL LOCATION

Genetic locus: UBE3A (human) mapping to 15q11-q13.

## SOURCE

E6-AP (EE-7) is a mouse monoclonal antibody raised against recombinant E6-AP of human origin.

## PRODUCT

Each vial contains 100 µg IgG<sub>2a</sub> in 1.0 ml PBS with < 0.1% sodium azide and 0.1% gelatin.

## STORAGE

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

## APPLICATIONS

E6-AP (EE-7) is recommended for detection of E6-AP of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for E6-AP siRNA (h): sc-43742, E6-AP shRNA Plasmid (h): sc-43742-SH and E6-AP shRNA (h) Lentiviral Particles: sc-43742-V.

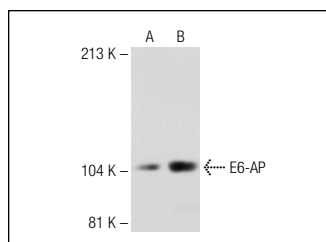
Molecular Weight of E6-AP: 100 kDa.

Positive Controls: IMR-32 nuclear extract: sc-2148 or Ramos nuclear extract: sc-2153.

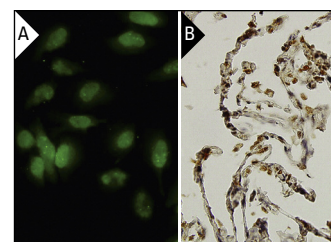
## RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 4) Immunohistochemistry: use ImmunoCruz™: sc-2050 or ABC: sc-2017 mouse IgG Staining Systems.

## DATA



E6-AP (EE-7): sc-100614. Western blot analysis of E6-AP expression in IMR-32 (A) and Ramos (B) nuclear extracts.



E6-AP (EE-7): sc-100614. Immunofluorescence staining of paraformaldehyde-fixed HeLa cells showing nuclear localization (A) and immunoperoxidase staining of formalin-fixed, paraffin-embedded human lung tissue showing nuclear and cytoplasmic localization (B).

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.