Technical Data Sheet

Purified Mouse Anti-Human p300

Material Number:	554215
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	NM11
Immunogen:	Human p300
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: primate Cos-7 cell lysate
	Cross-reactivity reported: human, monkey, mouse and rat
Target MW:	300 kDa
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

Many serotypes of human adenoviruses (Ad) are able to transform rodent cells in culture because of functions encoded by early region adenovirus 1A (E1A) and 1B (E1B) genes. These virus early gene products bind to specific cellular proteins that normally act to restrict cell growth. The E1A gene, essential for viral replication, encodes protein products with three amino acid regions (1, 2 and 3) conserved among all known adenovirus serotypes. Regions 1 and 2 and the relatively unconserved amino terminus (N) are E1A sequences required for cell growth-regulating functions. The region 2 site binds the retinoblastoma (Rb) and Rb related proteins, p107 and p130. The amino terminal site binds p300, a large cellular DNA binding protein. p300 is a relatively stable, ubiquitously expressed, nuclear phosphoprotein which is conserved among a variety of mammalian species. It is actively synthesized and phosphorylated in both quiescent and proliferating cells. A phosphatase sensitive form with decreased mobility has been identified in M-phase enriched cell populations. Evidence suggests that p300 plays a role in cellular transcription mechanisms. It has sequence-specific DNA binding activity with a consensus DNA binding sequence similar to enhancer elements targeted by the E1A repressor function. The presence of p300 and p300-associated proteins in complexes with the TATA binding protein suggests that p300 has specific interactions with basal transcription mechanisms. Through its association with the TATA binding rotein suggests that p300 has specific interactions with basal transcription mechanisms. TATA box sequences. p300 migrates at a reduced molecular weight at ~300 kD.

Clone NM11 recognizes human p300 a cellular E1A binding protein p300. Specifically, it has been shown to recognize p300 from human, monkey, mouse and rat cell lysates. NM11 will also coprecipitate E1A in association with p300 when E1A is present. Affinity purified p300 was used as immunogen. p300 was affinity purified from 293 human kidney cells (adenovirus type 5 [Ad5] transformed cell line) by co-immunoprecipitation using an E1A-specific monoclonal antibody (M73), which was covalently linked to protein A-Sepharose beads.



Immunoprecipitation of p300 and coprecipitation of E1A with NM11 (Cat. No. 554215). NM11 immunoprecipitated p300 from Cos-7 cells (lane 1), and both p300 and E1A from Cos-7 cells transfected with plasmids encoding either adenovirus (Ad)12 13S E1A (lane 2) or Ad12 12S E1A (lane 3) Cos-7 cells [35]S labeled.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4°C.

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Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Reported
Dot Blot	Reported

Recommended Assay Procedure:

Applications include Western blot and immunoprecipitation. Clone NMII is routinely tested by western blot analysis using the transfer protocol for high molecular weight proteins, please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml. Ad5 transformed cell lines including 293 cells (ATCC CRL 1573) are suggested as positive controls.

Product Notices

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Rikitake Y, Moran E. DNA-binding properties of the E1A-associated 300-kilodalton protein. *Mol Cell Biol.* 1992; 12(6):2826-2836.(Biology) Wang HG, Yaciuk P, Ricciardi RP, Green M, Yokoyama K, Moran E. The E1A products of oncogenic adenovirus serotype 12 include amino-terminally modified forms able to bind the retinoblastoma protein but not p300. *J Virol.* 1993; 67(8):4804-4813.(Clone-specific: Immunoprecipitation) Yaciuk P, Moran E. Analysis with specific polyclonal antiserum indicates that the E1A-associated 300-kDa product is a stable nuclear phosphoprotein that undergoes cell cycle phase-specific modification. *Mol Cell Biol.* 1991; 11(11):5389-5397.(Immunogen)