Technical Data Sheet

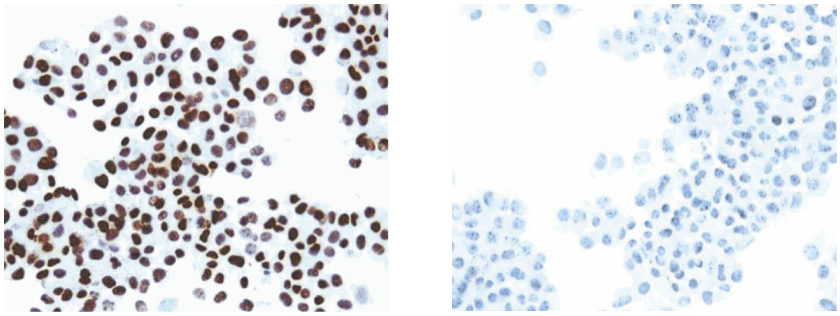
Purified Mouse Anti-SV40 Large T and Small t Antigens

Product Information

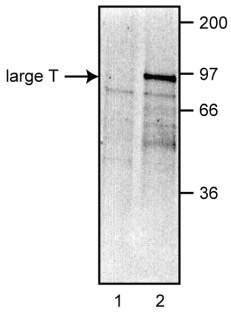
Material Number:	**554150**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	PAb 108
Immunogen:	SV40-transformed BALB/c mouse cells
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Viral-transformed cells
Target MW:	90-100 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Simian virus 40 (SV40) is a small DNA virus encoded by 5.2 kb of double-stranded DNA. SV40 large T-antigen (T-ag) is a multifunctional ~85 kDa phosphoprotein, which is the sole viral protein required for SV40 replication. All other factors are provided by the infected host cell. In addition to its role in SV40 DNA replication, T-ag also causes transformation of susceptible cell lines. Studies of various mutant T-ag proteins have shown that the replication and transformation fractions of T-ag can be separated. The multifunctional nature of this protein has resulted in its use as a model system in a wide variety of disciplines. T-ag exercises negative regulation on the transcription of SV40 early mRNA by feedback inhibition and exerts positive regulation on transcription from the late promoter. In addition to transcriptional regulation, T-ag is involved in viral DNA replication. Specific biochemical functions required for DNA synthesis that are inherent to the T-ag include high-affinity binding to sites within the viral origin of DNA synthesis, ATPase, and helicase activities. Other functions attributed to T-ag include cellular transformation, induction of cellular DNA synthesis, induction of rRNA synthesis, and provision of a host-range function for viral replication. However, functions of T-ag are influenced by a wide range of post-translational modifications including phosphorylation, glycosylation, acetylation, acylation, and adenylation. T-ag exists in monomeric as well as polymeric forms, and associates with the tumor suppressor proteins p53 and retinoblastoma protein (Rb). Most of T-ag is transported to the nucleus, while a small fraction is localized at the cell surface. Small t-Ag is a polypeptide which shares 82 N-terminal amino acids with large T antigen and has a unique C-terminal region.
Clone PAb 108 recognizes an N-terminal epitope within the first 82 amino acids of T-ag and small t antigen (t-ag). B4 SV40-transformed BALB/c mouse fibroblasts were used as immunogen. PAb 108 was originally produced and characterized as part of a panel of antibodies designated PAb 102-117.



Immunohistochemical staining of SV40 large T, small t antigen. *Cytospins of SV40-transformed COS-7 cells were acetone-fixed and stained with mouse anti-SV40 large T, small t antigen (Cat. No. 554151) (left panel) or with an isotype control (right panel). SV40 large T antigen staining is localized to the nucleus of the cells.*



Immunoprecipitation of large T from COS-7 cells. *Lane 1, a mouse IgG2a isotype control. Lane 2, PAb 108 (Cat. No. 554150).*

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
Immunofluorescence	Tested During Development
Immunoprecipitation	Tested During Development
Immunohistochemistry	Tested During Development

Recommended Assay Procedure:

By SDS-PAGE, T-ag migrates at 90-100 kDa and t-ag at 15-20 kDa. COS-7 kidney monkey cells (ATCC CRL 1651), other established SV40-transformed cell lines, SV40-virus infected cells, or cells transfected with SV40 DNA should be used as a positive control. Other applications include immunoprecipitation, Western blot analysis, immunofluorescence microscopy, immunohistochemical staining of acetone-fixed, tissue culture cells (cytospin) and SV40 origin DNA binding assays, although this application has not been tested at BD Pharmingen. The biotin-conjugated antibody, Cat. No. 554151 is recommended for cytospin staining and IHC applications.

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

Gurney EG, Tamowski S, Deppert W. Antigenic binding sites of monoclonal antibodies specific for simian virus 40 large T antigen. *J Virol.* 1986; 57(3):1168-1172. (Immunogen: Immunofluorescence, Immunoprecipitation, Western blot)

Hinzpeter M, Fanning E, Deppert W. A new sensitive target-bound DNA binding assay for SV40 large T antigen. *Virology.* 1986; 148(1):159-167. (Clone-specific: Immunohistochemistry, Immunoprecipitation)

Mellor A, Smith AE. Characterization of the amino-terminal tryptic peptide of simian virus 40 small-t and large-T antigens. *J Virol.* 1978; 28(3):992-996. (Biology)