

## Technical Data Sheet

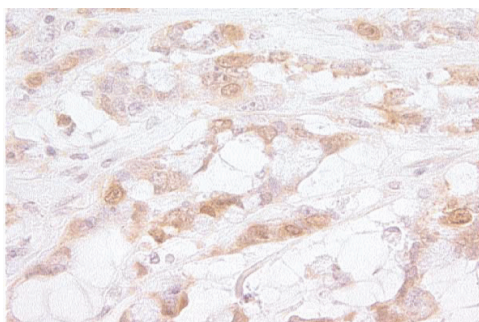
## Purified Mouse Anti-Human p16

## Product Information

Material Number:	550834
Alternate Name:	p16-INK4, p16-INK4a, ARF, MTS1, CDKN2, CDK4I
Size:	1 mL
Concentration:	31.25 µg/ml
Clone:	G175-405
Immunogen:	Human p16 Recombinant Protein
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA, goat serum, and ≤0.09% sodium azide.

## Description

Cyclins and cyclin-dependent kinases (cdks) form active complexes that regulate key events during the progression of the cell cycle and are evolutionarily highly conserved. The p16 protein has been identified as a specific inhibitor of cdk4 because it blocks cdk4 substrate phosphorylation. p16 inhibits cdk4 dependent phosphorylation of the tumor suppressor retinoblastoma protein (Rb) and Rb related proteins, p107 and p130. The biochemical properties of p16 suggest that it may be a tumor suppressor gene product. Recently a gene cloned from the short arm of human chromosome 9, Multiple Tumor Suppressor 1 (MTS1) has been identified as the gene for p16. The gene, now also known as the CDKN2 gene, has been found to be mutated in a very high percentage of tumors, including 75% of melanoma cell lines.



**Immunohistochemistry analysis of p16 expression on human breast tissue.** A formalin-fixed, paraffin-embedded section from human breast tissue was stained with the Mouse Anti-Human p16 antibody (Cat. No. 550834). Cells expressing p16 were visualized using a three-step staining procedure with Polyclonal Biotin Goat Anti-Mouse Ig (Cat. No. 550337) as the secondary antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB Substrate Kit (Cat. No. 550880). P16 expression can be identified by the intense brown labeling of cell nuclei (magnification 20X).

## Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

## Application Notes

## Application

Western blot	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development

## Recommended Assay Procedure:

**Immunohistochemistry:** The p16 antibody is recommended to test for immunohistochemical staining on formalin-fixed paraffin-embedded sections. Tissues tested were human breast or colon sections. For paraffin sections, microwave oven pretreatment with BD Retrieval A (pH 6.0) (Cat. No. 550524) is required. Staining is nuclear and/or cytoplasmic. Yeager et al. found p16 to be both nuclear and cytoplasmic in melanoma. The isotype control recommended for use with this antibody is purified mouse IgG1 (Cat. No. 550878). For optimal indirect immunohistochemical staining, this p16 antibody should be titrated (1:10 to 1:50 dilution) and visualized via a three-step staining procedure in combination with the Polyclonal Biotin Goat Anti-Mouse Ig (Cat. No. 550337) as the secondary antibody and Streptavidin-HRP (Cat. No. 550946) together with the DAB Substrate Kit (Cat. No. 550880). Alternatively, the Anti-Mouse Ig HRP Detection Kit (Cat. No. 551011) offers all the necessary reagents to stain for this antibody.

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550834 Rev. 4



## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
550524	Retrievagen A (pH 6.0)	1000 mL	(none)
550878	Purified Mouse IgG1 $\kappa$ Isotype Control	1 mL	MOPC-31C
551011	Anti-Mouse Ig HRP Detection Kit	200 Tests	(none)
550337	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.25 mg	Polyclonal
550946	Streptavidin HRP	50 mL	(none)
550880	DAB Substrate Kit	500 Tests	(none)
559148	Antibody Diluent for IHC	125 mL	(none)
551153	Purified Mouse Anti-Human p16 with Control	50 $\mu$ g	G175-405
551154	Purified Mouse Anti-Human p16 with Control	150 $\mu$ g	G175-405

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. 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.
5. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
6. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
7. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

## References

Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science*. 1994; 264(5157):436-440. (Biology)

Marx J. Link to hereditary melanoma brightens mood for p16 gene. *Science*. 1994; 265(5177):1364-1365. (Biology)

Serrano M, Hannon GJ, Beach . A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*. 1993; 366(6456):704-707. (Immunogen)

Yeager T, Stadler W, Belair C, Puthenveetil J, Olopade O, Reznikoff C. Increased p16 levels correlate with pRb alterations in human urothelial cells. *Cancer Res*. 1995; 55(3):493-497. (Clone-specific)