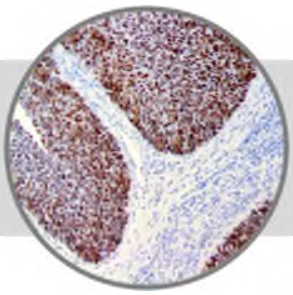
PI 3600, Rev. A DCN: 3682

p14 ARF / CDKN2A, RMab

Clone: RBT-p14

Rabbit Monoclonal



IVD

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Inset: IHC of p14 ARF on a FFPE Anal Carcinoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Synthetic peptide corresponding to the C-terminus of the human p14 ARF/CDKN2A.

Summary and Explanation

p14 ARF (also called ARF tumor suppressor, ARF, p14ARF) encoded by the p16 tumor suppressor gene is an alternate reading frame protein product of the CDKN2A locus (i.e. INK4a/ARF locus). p14ARF accumulates mainly in the nucleolus where it forms stable complexes with NPM or MDM2. These interactions allow p14ARF to act as a tumor suppressor by inhibiting ribosome biogenesis or initiating p53-dependent cell cycle arrest and apoptosis, respectively. Both p16INK4a and p14ARF are involved in cell cycle regulation. p14ARF inhibits MDM2, thus promoting p53, which promotes p21 activation, which then binds and inactivates certain cyclin-CDK complexes, which would otherwise promote transcription of genes that would carry the cell through the G1/S checkpoint of the cell cycle. Loss of p14ARF by a homozygous mutation in the CDKN2A (INK4A) gene will lead to elevated levels in MDM2 and, therefore, loss of p53 function and cell cycle control.

p14ARF, has been reported to be associated with the clinicopathological features of different cancers. Very commonly, cancer is associated with a loss of function of INK4a, ARF, Rb, or p53. Without ARF, MDM2 can inappropriately inhibit p53, leading to increased cell survival. The INK4a/ARF locus is found to be deleted or silenced in many kinds of tumors. It has been found that 41% breast carcinomas have p14ARF defects and in a separate study, 32% of colorectal adenomas were found to have p14ARF inactivation due to hypermethylation of the promoter. Homozygous deletions and other mutations of CDK2NA (ARF) have been found to be associated with Glioblastoma. p14ARF expression has been found to be significantly associated with the risk of lung cancer.

Antibody Type	Rabbit Monoclonal	**Clone**	RBT-p14
Isotype	IgG	**Reactivity**	Paraffin, Frozen
Localization	Nuclear, Cytoplasmic	**Control**	Cervical, Anal and Ovarian Carcinomas
Species Reactivity		Human	

Presentation

P14 ARF is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Dilution*	*Volume/Qty*
BSB 3595	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3596	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3597	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3598	Concentrated	1:25 - 1:100	0.1 mL
BSB 3599	Concentrated	1:25 - 1:100	0.5 mL
BSB 3600	Concentrated	1:25 - 1:100	1.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB 3601	5 slides

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Storage Store at 2-8^{0}C (Control Slides: Store at 20-25^{0}C)

Stability

This product is stable up to the expiration date on the product label. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with Immuno/DNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

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2. Abida WM, Gu W (January 2008). "p53-Dependent and p53-independent activation of autophagy by ARF". Cancer Res. 2008; 68 (2): 352–7
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4. Lowe SW, Sherr CJ. "Tumor suppression by Ink4a-Arf: progress and puzzles". Curr. Opin. Genet. Dev. 2003; 13 (1): 77–83.
5. Yi Y, Shepard A, Kittrell F, Mulac-Jericevic B, Medina D, Said TK (May 2004). "p19ARF Determines the Balance between Normal Cell Proliferation Rate and Apoptosis during Mammary Gland Development". Mol. Biol. Cell. 2004; 15 (5): 2302–11.
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Symbol Key / Légende des symboles/Erläuterung der Symbole

	Storage Temperature Limites de température Zulässiger Temperaturbereich	Manufacturer Fabricant Hersteller	REF Catalog Number Référence du catalogue Bestellnummer
IVD In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	Expiration Date Utiliser jusque Verwendbar bis	LOT Lot Number Code du lot Chargenbezeichnung

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