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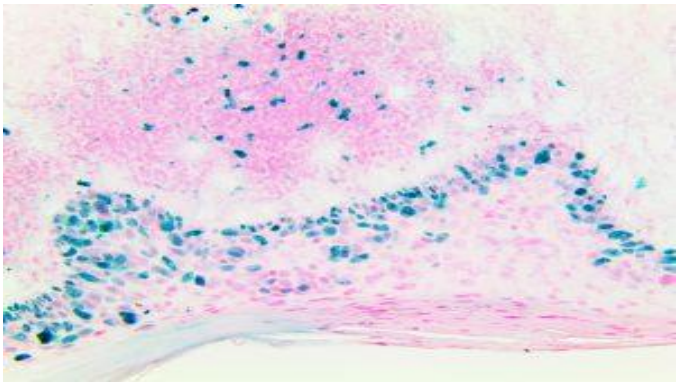
# TintoFast Ki-67

**Clone:** RM360
Rabbit Monoclonal

CE IVD



*Inset: IHC of TintoFast Ki-67 on a Frozen Squamous Cell Carcinoma Tissue*

## Intended Use
For Mohs In Vitro Diagnostic Use.

This antibody is intended for the fast immunohistochemical detection of Ki-67, a protein associated with cell proliferation, during intraoperative Mohs surgery on frozen sections. Additionally, this antibody can also be used on FFPE specimens. Interpretation of results should be performed by a qualified medical professional.

## Immunogen
A peptide corresponding to the internal region of human Ki67.

## Summary and Explanation
The Ki-67 protein is a cellular marker for proliferation. It is strictly associated with cell proliferation. During the interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0).

Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer. The best-studied examples in this context are carcinomas of the prostate and the breast.

| | | | |
|---|---|---|---|
| **Antibody Type** | Rabbit Monoclonal | **Clone** | RM360 |
| **Isotype** | IgG | **Reactivity** | Paraffin, Frozen |
| **Localization** | Nuclear | **Species Reactivity** | Human |
| **Control** | Skin | | |
| **Application** | Mohs, Melanoma & Skin Cancer | | |

## Presentation
Anti-TintoFast Ki-67 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.* | *Presentation* | *Dilution* | *Volume* |
|---|---|---|---|
| BSB-3771-3 | TintoFast Predilute | Ready-to-Use | 3.0 mL |
| BSB-3771-7 | TintoFast Predilute | Ready-to-Use | 7.0 mL |
| BSB-3771-15 | TintoFast Predilute | Ready-to-Use | 15.0 mL |

## 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 a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts 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 (BSB 7008), TintoDetector Incubator (BSB 7002) 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).

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

## Mohs IHC Protocol
### Specimen Preparation of Mohs Frozen Tissues
1. Embed the specimen in OCT inside a cryostat.
2. Cut sections at 4-5 µm and mount on a positively charged glass slide such as the Bio SB Hydrophilic Plus Slides (BSB 7028) or TintoDetector Cap Gap Plus slides (BSB 7006).
3. Air dry the slide at room temperature for 2 minutes and then incubate the slide at 60 °C for 3 minutes in an incubator or dry bath.
4. Fix in 100% acetone for 2 minutes at room temperature and let the slide air dry.

### Tissue Pretreatment Procedure for Mohs Frozen Tissues
Subject tissues to HIER (heat-induced epitope retrieval) using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023).

**a. For Mohs PolyDetector HRP Green or DAB protocol use the TintoRetriever Pressure Cooker (BSB 7008) or Equivalent.** Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate, and place on trivet or staining dish support in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high (110-121° C). Incubate for 5 minutes. Open and immediately transfer slides to room temperature. Cool off for 5 -10 min

**b. For Mohs PolyDetector Plus HRP Green or DAB protocol use the TintoDetector Incubator (BSB 7002).** Preheat the TintoDetector Incubator to 110 °C. Place TintoDetector Cap Gap slides (BSB 7006) face to face and insert them into the TintoDetector Slide Holder (BSB 7003). Submerge slides in ImmunoDNA Retriever with Citrate to draw up enough solution by capillary action to cover the tissues. Heat the slides in a preheated TintoDetector Incubator for 3 minutes. Transfer slides to room temperature and cool off for 1 min.

### Mohs IHC Detection

1. After HIER, transfer slides to ImmunoDNA washer and let it stand for 1-2 minutes.
2. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
3. Wash slides with ImmunoDNA Washer or DI water.
4. Continue IHC detection protocol. Wash slides between each step with ImmunoDNA washer solution.

### Abbreviated Mohs Immunohistochemical Protocol*

| Step | Mohs PolyDetecto HRP Green or DAB 10 min* Protocol | Mohs PolyDetecto Plus HRP Green or DAB 20 min Protocol |
|---|---|---|
| HIER | 5 min | 3 min |
| Primary Antibody | 4 min. | 5 min. |
| 1st Step Detection | 3 min. | 4 mn. |
| 2nd Step Detection | NA. | 4 min. |
| Substrate- Chromogen | 2 min. | 1-2 min. |
| Counterstain / Coverslip | Varies | Varies |

*instrument setup and HIER time not included

### IHC Protocol for FFPE Tissues

**Specimen Preparation of FFPE Tissues**

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides.
2. Air dry for 2 hours at 58° C.

**Tissue Pretreatment Procedure for FFPE Tissues**

1. Deparaffinize, dehydrate, and rehydrate tissues.
2. Subject tissues to HIER using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate or EDTA.
3. 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 10- 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 20-30 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 20-30 minutes.

1. After HIER, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 10 minutes.
2. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
3. Wash slides with ImmunoDNA washer or DI water.
4. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

### Mounting Protocols

Mount with aqueous media such as AquaMounter (BSB-0090- BSB 0093) or apply Fast ChromoProtector (BSB 0327) and then permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent-based resin such as PermaMounter (BSB 0094-0097).

### 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


1. Stoehlmacker J, Lenz Heinz-Josef, Semin Oncol. 2003;30(3)suppl6(June):10-16
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3. Sano H, et al. Cancer Res. 1995;Sep;55(17):3785-9
4. Denkert C, et al. Cancer. 2003;Jun15;97(12):2978-87
5. Sheehan KM, et al. Hum Pathol. 34:1242-1246
6. Birner P, Ritzi MJ, Voigtländer T, et al. Am J Pathol. 2001;158:1991-6
7. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe WorkPractices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf


### IHC Detection

### Symbol Key / Légende des symboles/Erläuterung der Symbole

| | | | |
|---|---|---|---|
| EC REP<br>EMERGO EUROPE<br>Prinsessegracht 20<br>2514 AP The Hague<br>The Netherlands | Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich | Manufacturer<br>Fabricant<br>Hersteller | REF<br>Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| IVD<br>In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum | Read Instructions for Use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | Expiration Date<br>Utiliser jusque<br>Verwendbar bis | LOT<br>Lot Number<br>Code du lot<br>Chargenbezeichnung |


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