

# TintoFast EpCAM BerEP4

Clone: Ber-EP4

Mouse Monoclonal



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*Inset: IHC of EpCAM BerEP4 on a Frozen Acetone-Fixed Extramammary Paget's Disease Tissue*

## Intended Use

For Research Use Only.

This antibody is intended for the fast immunohistochemical detection of EpCAM positive cells for 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

MCF-7 human breast carcinoma cell line.

## Summary and Explanation

Epithelial Cell Adhesion Molecule (EpCAM) or Epithelial Specific Antigen is a 40kD cell surface antigen that is broadly distributed in epithelial cells and displays a highly conserved expression in carcinomas. These glycoproteins are located on the cell membrane surface and in the cytoplasm of virtually all epithelial cells, with the exception of most squamous epithelia, hepatocytes, renal proximal tubular cells, gastric parietal cells and myoepithelial cells. However, focal positivity may be seen in the basal layer of squamous cell epithelium of endoderm (e.g., palatine tonsils) and mesoderm (e.g., uterine cervix).

EpCAM expression has been reported to be a possible marker of early malignancy, with expression being increased in tumor cells, and de novo expression being seen in dysplastic squamous epithelium. Epithelial specific antigen has been known to play an important role as a tumor-cell marker in lymph nodes from patients with esophageal carcinoma. EpCAM can be used to distinguish among Basal Cell, Basosquamous Carcinomas and Squamous Cell Carcinomas of the skin.

<b>Antibody Type</b>	Mouse Monoclonal	<b>Clone</b>	Ber-EP4
<b>Isotype</b>	IgG1/K	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Cytoplasmic	<b>Control</b>	BCC, EMPD
<b>Species Reactivity</b>		Human	

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN<sub>3</sub>) 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).

## Presentation

Anti - EpCAM is a mouse 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 3678	TintoFast Prediluted	Ready-to-Use	3.0 mL
BSB 3679	TintoFast Prediluted	Ready-to-Use	7.0 mL
BSB 3680	TintoFast Prediluted	Ready-to-Use	15.0 mL

**Storage** Store at 2-8°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.

## 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.

## Specimen Preparation

**Mohs Frozen sections and cell preparations:** The antibody can be used for the detection of EpCAM antigen in Mohs acetone-fixed frozen sections.

**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). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

## Mohs IHC Procedure

### 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).
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.
5. Rinse with distilled water and air dry the slides for another 2 minutes at room temperature.

## Tissue Pretreatment Procedure for Mohs Frozen Tissues

1. Subject tissues to epitope retrieval using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). It is recommended that user uses a **5 minutes** heat-induced epitope retrieval (HIER) method using a pressure cooker (BSB TintoRetriever Pressure Cooker) at 110°C.

2. Any of three HIER 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 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. 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 10-15 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 10-15 minutes.

## IHC Detection Procedure

1. After HIER, transfer slides to ImmunoDNA washer and let 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

For best results we recommend using Mohs Frozen sections fixed with acetone for 2 min. then HIER with Citrate for 5 min at 110-121 °C.

Step	Mohs PolyDetector HRP Green or DAB 5 min Protocol	Mohs PolyDetector HRP Green or DAB 10 min Protocol
Peroxidase/AP Blocker	0.5 min.	0.5 min.
Primary Antibody	2 min	4 min.
1st Step Detection	1 min	3 min.
Substrate-Chromogen	1 min	2 min.
Counterstain / Coverslip	0.5 min	0.5 min.

This protocol can also be used with FFPE Tissues retrieved with Citrate or EDTA.

## Specimen Preparation for 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 (BSB 7028).
2. Air dry for 2 hours at 58° C.

## Tissue Pretreatment Procedure for FFPE Tissues

1. Deparaffinize and rehydrate tissues.
2. 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).

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.

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

## Mounting Protocols

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

## References

1. Beer TW, et al. Histopathology. 2000;37:218-223
2. Carella R, et al. Am J Surg Pathol. 2001;25:43-50
3. Hosch S, et al. Cancer Res. 2000; 60:6836-6840
4. Latza U, et al. J Clin Pathol. 1990;43:213-219
5. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

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

	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung



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