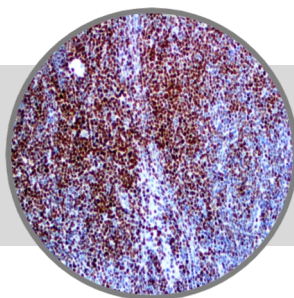


# Histone H3 Phospho, RMAb

**Clone: EP233**

Rabbit Monoclonal



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*Inset: IHC of Histone H3 Phospho on a FFPE NH Lymphoma 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.

\* The Histone H3 Phospho antibody, clone EP233, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

## Immunogen

A synthetic phospho-peptide corresponding to residues surrounding Thr3 of Human Histone H3.

## Summary and Explanation

Phosphohistone-H3 is one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N-terminal tail, H3 is involved with the structure of the nucleosomes of the "beads on a string" structure. In mammalian cells, phosphohistone H3 is negligible during interphase but reaches a maximum for chromatin condensation during mitosis.

Phosphohistone-H3 can serve as a mitotic marker to separate mitotic figures from apoptotic bodies and karyorrhectic debris, which may be a very useful tool in diagnosis of tumor grades, especially in CNS, skin, Gyn., soft tissue, and GIST.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	EP233
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Nuclear	<b>Control</b>	Tonsil, Lymph Node
<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

Histone H3 Phospho 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.

<i>Catalog No.</i>	<i>Antibody Type</i>	<i>Dilution</i>	<i>Volume/Qty</i>
BSB 2524	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2525	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2526	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2527	Concentrated	1:100 - 1:500	0.1 mL
BSB 2528	Concentrated	1:100 - 1:500	0.5 mL
BSB 2529	Concentrated	1:100 - 1:500	1.0 mL

## Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 2530	5 slides

**Storage** Store at 2-8°C (Control Slides: Store at 20-25°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, dehydrate 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 ImmunoDNA 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

Normal Tissues	
Positive (+)	
Tonsil	15/15 (100%)
Lymph Node	5/5 (100%)
Breast	5/5 (100%)
Negative (-)	
Liver	0/5 (0%)
Prostate	0/5 (0%)
Abnormal Tissues	
Positive (+)	
Melanoma	86/113 (76%)
Lymphoma	6/8 (75%)





## 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. Bhasin M, et al. J Comput Biol. 2006; 13(1):102-12
2. Colman H, et al.: Am J Surg Pathol 2006, 30:657-664
3. Hightower E, et al.: Pituitary 2012, 15:556-561
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5. Nasr MR, et al. Am J Dermatopathol. 2008; 30:117-22
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7. Yang GG: MLO Med Lab Obs 2011, 43:20, 23
8. 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

<b>EC REP</b>	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
<b>IVD</b>	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	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung