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Inset: IHC of GATA3 on a FFPE Breast Carcinoma Tissue

# **Intended** Use

For In Vitro Diagnostic Use.

GATA3, RMab

Clone: EP368 Rabbit Monoclonal

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

\* The GATA3 antibody, clone EP368, has been manufactured using Epitomics RabMab<sup>®</sup> technology covered under Patent No.'s 5,675,063 and 7,402,409.

### Immunogen

Synthetic peptide corresponding to residues of human GATA3 protein.

### **Summary and Explanation**

Trans-acting T-cell-specific transcription factor, GATA-3 is, a protein that in humans is encoded by the GATA3 gene. GATA-3 b regulates luminal epithelial cell differentiation in the mammary gland, is an important regulator of T cell development and plays an important role in endothelial cell biology.

GATA-3 is one of the three genes mutated in >10% of breast cancers. Nuclear expression of GATA-3 in breast cancer is considered a marker of luminal cancer in ER+ cancer and luminal androgen responsive cancer in ER-/AR+ tumors. It is highly coexpressed with FOXA1 and serves as negative predictor of basal subtype and HER-2 and is also considered a strong predictor of taxane and platin salts insensitivity.

GATA3 expression is found in urothelial carcinoma, especially in invasive and high grade tumors. Therefore, anti-GATA3 can be used in a panel of antibodies for diagnosis of unknown primary carcinoma, when carcinomas of the breast or bladder are a possibility. Studies have also shown the utility of GATA-3 in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine, cervix, anus and lung.

Antibody Type	Rabbit Monoclonal	Clone	EP368		
lsotype	lgG	Reactivity	Paraffin, Frozen		
Localization	Nuclear	Control	Breast, Skin, Cervix, Breast Carcinoma		
	Species Reactivity	Human			

### Presentation

GATA-3 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.

C€IVD

Catalog No.	Antibody Type	Dilution	Volume/Qty
BSB 3328	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3329	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3330	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3331	Concentrated	1:50 - 1:200	0.1 mL
BSB 3332	Concentrated	1:50 - 1:200	0.5 mL
BSB 3333	Concentrated	1:50 - 1:200	1.0 mL

### **Control Slides Available**

Catalog No.	Quantity		
BSB 3334	5 slides		

**Storage** Store at 2-8°C (Control Slides: Store at 20-25°C)

### 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).

### 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**

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. Yamashita M, et al. . Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. 2004; J Biol Chem 279 (26): 26983–90.

 Wilson BJ, Giguere V. Meta-analysis of human cancer microarrays reveals that GATA3 is integral to the estrogen receptor alpha pathway. Mol Cancer 7: 49.
Dydensborg AB, et al. GATA3 inhibits breast cancer growth and pulmonary breast

cancer metastasis. Oncogene 2009; 28 (29): 2634–42.

4. Sanga S, et al. Gene expression meta-analysis supports existence of molecular apocrine breast cancer with a role for androgen receptor and implies interactions with ErbB family. BMC Medical Genomics 2009; 2: 59.

5. Higgins JP, et al. Placental S100 (S100P) and GATA3: Markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol. 2007;31:673–680.

6. Liu, H, et al. Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol 2012;138:57-64.

7. 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

	EMERGO EUROPE Prinsessegracht 20 514 AP The Hague The Netherlands	A.C	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
Dispositif médical de	tic Medical Device diagnostic in vitro itro-Diagnostikum	Ĩi	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	$\sum$	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung
Bio SB							

