

Anti-TIGIT / DIA-TG2-M

Mouse monoclonal anti-T cell marker
(Immune checkpoint protein) Clone TG2

Product Information

Cat.No.:	DIA-TG2-M	Species Reactivity:	Human
Clone:	TG2	Positive Control:	Tonsil
Specificity:	Human TIGIT	Visualization:	Membranous
Isotype:	Mouse IgG1/k	Manufacturer:	ONCOdianova GmbH
Immunogen:	Recombinant peptide from extracellular domain of human TIGIT	Reconstitution:	DIA-TG2-M, restore to 100 µl with sterile distilled water by gentle shaking for 10 minutes
Physical state:	Lyophilized powder	Presentation:	In PBS with 2% BSA, 0.05% NaN ₃ , pH 7.4. Antibody purified from culture supernatant
Dilutions:	1:100–1:200 for polymer-based detection 1:50 for biotin/(strept)avidin-based detection	Applications:	Immunohistochemistry (IHC), standard formalin-fixed paraffin sections

The dilutions stated are general starting recommendations. Validation of antibody performance and protocol is the responsibility of the end user. Positive and negative controls should be run simultaneously with the specimen.

1. Procedure — Manual Staining, IHC FFPE tissue

SLIDE PREPARATION

- mount 4 µm sections on superfrost slides
- deparaffinize tissue sections in xylene (2× 5 min)
- rehydrate in descending ethanol series (100 %, 90 %, 80 %, 70 %)
- rinse 5 minutes in TBST* buffer

PRETREATMENT (EPI TOPE RETRIEVAL)

- autoclave for 5 min** at 121 °C in TEC*** buffer pH 7.8 (1× concentration)
- rinse 5 minutes in TBST* buffer

PEROXIDASE-BLOCKING

- incubate 10 min in Peroxidase-Blocking Solution (#S2023, DAKO REAL™)
- rinse 2× 5 min in TBST* buffer

ANTIBODY INCUBATION

- dilute primary antibody (anti-TIGIT, clone TG2, DIA-TG2-M) 1:100–1:200 in antibody diluent (#S2022, DAKO REAL™) — polymer-based detection

- cover tissue section with 100–200 µl diluted antibody
- incubate 1 h at 37 °C in moist chamber
- rinse 2× 5 min in TBST* buffer
- apply DAKO EnVision Polymer-HRP mouse/rabbit Kit (#K5007, DAKO REAL™) according to manufacturer's recommendation
- rinse 2× 5 min in TBST* buffer

CHROMOGEN

- cover slides for 10 min with DAB-Chromogen (DAKO EnVision Polymer-HRP mouse/rabbit Kit, #K5007, DAKO REAL™)
- wash slides in H₂O dest. thoroughly
- counterstain for 20 sec with Hematoxylin (Mayer's Hematoxylin 41-5131-00, Medite GmbH)
- develop for 5 min in water
- dehydrate in ascending ethanol series
- wash in xylene
- apply mounting medium and coverslips

* TBST wash buffer (#K8000, DAKO)

** Incubate 5 min after autoclave has reached 121 °C

*** TEC: Stock solution = 20× Tris-EDTA-citrate buffer: per 1 liter aqua dest. dissolve 5 g Trizma base (Sigma T 1503), 10 g EDTA (Merck 1.08418.0250), 6.4 g tri sodium citrate (Sigma C 0909), adjust to pH 7.8 using HCl 1 mol.

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2. Procedure — Automated Staining, Leica Bond RX

BEFORE LOADING THE SYSTEM

- dewax & rehydrate sections
- heat pretreatment before entering the slides into the system: HIER (heat induced epitope retrieval), high pH buffer, pH 9.0, 100 °C, 20 min (alternatively: autoclave at 121 °C, 5 min)
- cool slides

LEICA BOND RX PROTOCOL

- rinse with Bond wash solution 3×
- peroxide block: Leica Microsystems, 5 min
- rinse with Bond wash solution 3×
- **primary antibody: anti-TIGIT / clone TG2 (DIA-TG2-M), 1:100–1:200, 37 °C, 60 min**
- rinse with Bond wash solution 3×
- Post Primary (Leica Microsystems), 30 min
- rinse with Bond wash solution 3× 2 min
- Polymer (Leica Microsystems), 30 min
- Bond wash solution 2× 2 min; deionized water 1×
- Mixed DAB Refine, 10 min
- deionized water 3×
- Hematoxylin (Leica Microsystems), 5 min
- deionized water 1×, Bond wash solution 1×, deionized water 1×; mount

Dispense volume 150 µl per step.

Expected result

- the antibody stains cell membranes of various lymphocyte subtypes
- weak non-specific nuclear/nucleolar staining may occur in some epithelial tissues (i.e. colon cancer)

Storage and Stability

Store the lyophilized antibody at 2–8 °C. For long term storage freeze at –20 °C, thus the antibody is stable for at least one year. As reconstituted liquid store at 2–8 °C short term (several weeks). Avoid repeated freeze / thaw cycles.

Safety Notes

The material contains 0.05% sodium azide as preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material. Avoid skin and eye contact, inhalation and ingestion.

For Research Use Only. Not for use in diagnostic procedures.

Changes of the original product formulation or composition for commercial use are expressly prohibited.

