
Anti-TIGIT / DIA-TG1

Mouse monoclonal anti-T cell marker (Immune checkpoint protein) Clone TG1

1. Procedure	Manual Staining IHC FFPE tissue	Cat.No: Clone: Specificity: Isotype: Concentration: Physical state: Reconstitution:	DIA-TG1, DIA-TG1-M TG1 Human TIGIT Mouse IgG1/k 0.4 mg/ml Lyophilized powder DIA-TG1, restore to 500 µl, DIA-TG1-M, restore to 100 µl with sterile distilled water by gentle shaking for 10 minutes
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SLIDE PREPARATION

- mount 4µm sections on superfrost slides
- deparaffinize tissue sections in xylene (2x 5min)
- 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 pH7.8 (1x concentration)
- rinse 5 minutes in TBST* buffer

PEROXIDASE-BLOCKING

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

ANTIBODY INCUBATION

- dilute primary antibody (TG-1, stock 0.4mg/ml) 1:50 – 1:150 in antibody diluent (#S2022, DAKO REAL[™])
- cover tissue section with 100-200µl diluted antibody
- incubate 1h at 37°C in moist chamber
- rinse 2x 5 min in TBST* buffer
- apply DAKO EnVision Polymer-HRP mouse/rabbit Kit (#K5007, DAKO REAL[™]) according to manufacturer's recommendation
- rinse 2x 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 mit 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 = 20x Tris-EDTA-citrate buffer: per 1 liter aqua dest. dissolve 5g Trizma base (Sigma T 1503), 10g EDTA (Merck 1.08418.0250), 6.4g tri sodium citrate (Sigma C 0909), adjust to pH 7.8 using HCL 1mol.



Fig.1: Microphotographs of dianova's TG-1 immunostaining on human tonsil

Antibody dilution

1:50

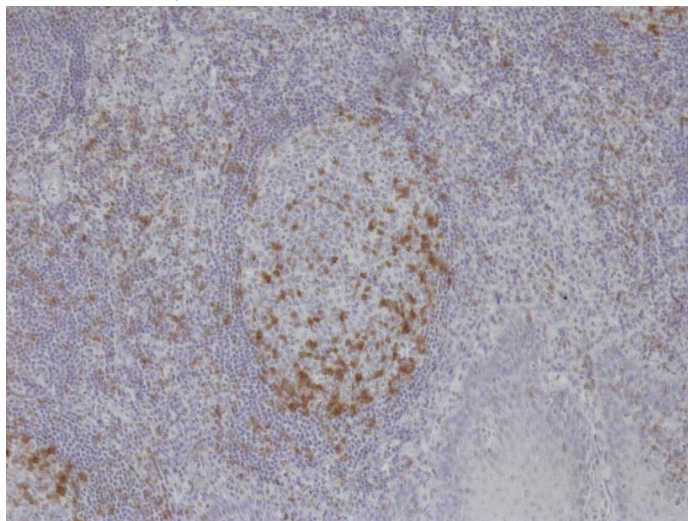
Antigen retrieval

Autoclave in TEC*-buffer (pH7.8) at 121°C for 5 min

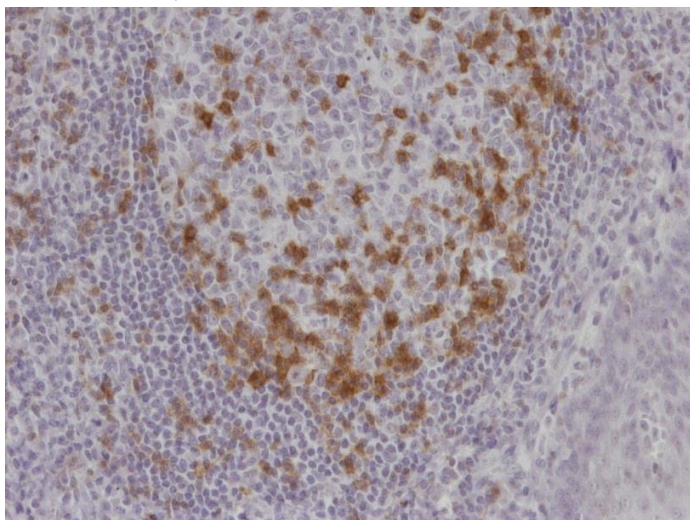
*Tris-EDTA-Citrate

Images taken with Zeiss Axiocam MRc camera and AxioVision SE64 software, fitted to a Zeiss Axioskope 40 microscope. No image enhancement or manipulation.

Human tonsil, 100x



Human tonsil, 200x



Human tonsil, 400x

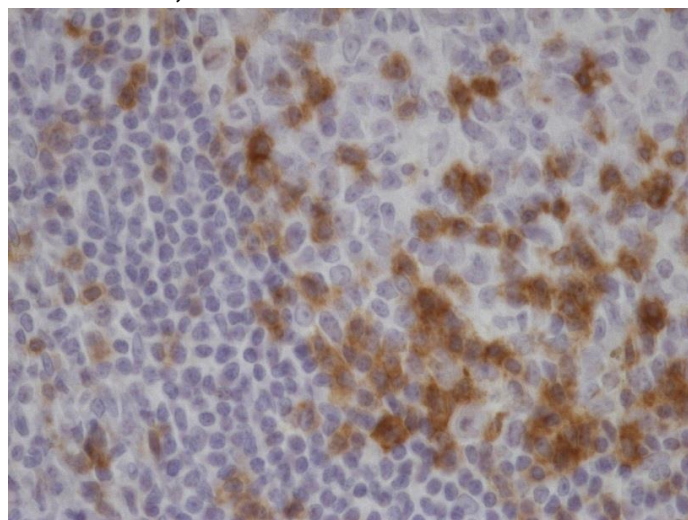
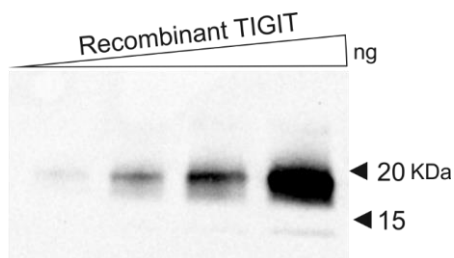


Fig. 2: Western blot using recombinant TIGIT protein as a target for dianova TG-1

a-TIGIT (Dianova) 1:500 5% milk PBST



Anti-TIGIT / DIA-TG1

Mouse monoclonal anti-T cell marker (Immune checkpoint protein) Clone TG1

2. Procedure	Automated Staining IHC FFPE tissue	Cat.No:	DIA-TG1 (200µg)
	Leica Bond RX	Clone: Specificity: Isotype: Concentration: Physical state: Reconstitution:	TG1 Human TIGIT Mouse IgG1/k 0.4 mg/ml Lyophilized powder DIA-TG1 (200µg), restore to 500 µl with sterile distilled water by gentle shaking for 10 minutes

BOND RX

Step	Reagent	<i>Supplier: Leica Microsystems</i>		
1	*Peroxide Block			
Step type:	Reagent	Inc. (min): 5:00	Temperature: Ambient	Dispense type: 150 µL
2	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
3	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: Open
4	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
5	Primary			
Step type:	Reagent	Inc. (min): 60:00	Temperature: 37	Dispense type: 150 µL
6	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
7	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
8	*Bond Wash Solution			
Step type:	Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL



Step Reagent				<i>Supplier: Leica Microsystems</i>
9 *Post Primary				
Step type: Reagent	Inc. (min): 30:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
10 *Bond Wash Solution				
Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
11 *Bond Wash Solution				
Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
12 *Bond Wash Solution				
Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
13 *Polymer				
Step type: Reagent	Inc. (min): 30:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
14 *Bond Wash Solution				
Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
15 *Bond Wash Solution				
Step type: Wash	Inc. (min): 2:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Not applicable</i>
16 *Deionized Water				
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
17 *Mixed DAB Refine				
Step type: Reagent	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Leica Microsystems</i>
18 *Mixed DAB Refine				
Step type: Reagent	Inc. (min): 10:00	Temperature: Ambient	Dispense type: 150 µL	
Step Reagent				<i>Supplier: Not applicable</i>
19 *Deionized Water				
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL	

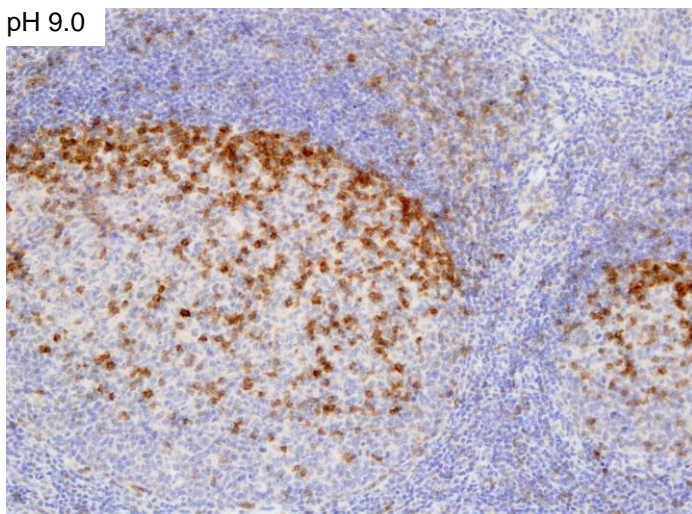


Step Reagent			<i>Supplier: Not applicable</i>
20 *Deionized Water			
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent			<i>Supplier: Not applicable</i>
21 *Deionized Water			
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent			<i>Supplier: Leica Microsystems</i>
22 *Hematoxylin			
Step type: Reagent	Inc. (min): 5:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent			<i>Supplier: Not applicable</i>
23 *Deionized Water			
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent			<i>Supplier: Leica Microsystems</i>
24 *Bond Wash Solution			
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL
Step Reagent			<i>Supplier: Not applicable</i>
25 *Deionized Water			
Step type: Wash	Inc. (min): 0:00	Temperature: Ambient	Dispense type: 150 µL

Antigen retrieval before entering the slide into the system.
HIER (heat induced epitope retrieval) ER2 is high pH of 9.0 at 100 degrees Celsius for 20min.

TIGIT IHC on Tonsil

pH 9.0



pH 6.0

