

## Anti - CD3 – rabbit clonal antibody

Cat. #: DB 082-0.1 (100 µl)      DB 082-1 (1 ml)  
 DB 082-0.5 (500 µl)      DB 082-T (test sample)

### Product information

<b>Clone:</b> N26-R	<b>Preservative:</b> 0.05% NaN <sub>3</sub>
<b>Buffer:</b> 20 mM Tris-HCl, pH 8.0	<b>Specificity:</b> Human
<b>Stabilizer:</b> 20 mg/ml BSA	<b>Storage:</b> -20°C
<b>Protein accession number:</b> P07766	<b>Expiration:</b> 18 months from the day of delivery

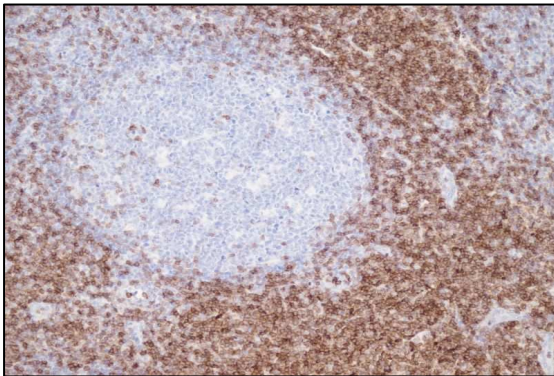
**Immunogen:** Peptide derived from cytoplasmic, C-terminal region of human CD3-epsilon chain

**Application:** IHC-P **dilution 1:100 - 200**; IHC-fr dilution to be tested by the user

**Centrifuge the vial before use**

### IHC-P Protocol

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% benzyl alcohol for 5 minutes each.
3. Rinse in distilled water.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 10 minutes.
5. Wash in distilled water.
6. For antigen retrieval: immerse the slide in Tris-EDTA buffer, pH 9.0, 0.05% Tween- 20\*, and incubate in microwave (850W) for 20 minutes. (*Alternatively adjust to your own protocol, keeping the required pH*)
7. Remove the staining to room temperature and let the slide to cool for 15 minutes.
8. Rinse in distilled water.
9. Wash in 0.05 M Tris-HCl , pH 7.6 buffer supplemented with 0.2% of Tween-20 (*buffer A*) for 5 minutes.
10. Incubate the section with primary antibody diluted in *buffer A* at the **dilution 1:100 - 200** for 1 hour in the closed wet chamber.
11. Wash twice 5 minutes with *buffer A*.
12. Apply the secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol (HRP – Peroxide – DAB).
13. Wash twice 5 minutes with *buffer A*.
14. Apply the chromogen (DAB), 10 minutes.
15. Wash in water – 10 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water – 10 minutes.
18. Dehydrate the section in 2 changes of 96% benzyl alcohol for 5 minutes each.
19. Wash the section in 2 changes of xylene for 2 minutes each.
20. Mount the slide for observation.



Formalin-fixed and paraffin-embedded human tonsil tissue (4 µm) stained with anti-CD3 (DB 082) antibody shows strong immunostaining of T-lymphocytes.

Kindly performed and provided by Katarína Poliaková, MD and Ľubomír Straka, MD, Ph. D. from Clinical Pathology Prešov, Ltd., Prešov, Slovak republic.

**\* Tris-EDTA Buffer (10mM Tris Base, 1mM EDTA solution, 0.05% Tween 20, pH 9.0):**

TRIS ----- 1.21 g; EDTA ----- 0,37 g

Distilled water ----- 1000 ml

Mix to dissolve in 700 ml of distilled water. Adjust pH to 9.0 with 1M HCl and then add 0.5 ml of Tween-20 and mix well. Adjust the final volume to 1 liter with distilled water.

Store this solution at room temperature for 3 months or at +4°C for longer storage.