

Anti - p60 – rabbit clonal antibody

Cat. #: DB 203-0.1 (100 µl) DB 203-0.5 (500 µl)
 DB 203-0.2 (200 µl) DB 203-1 (1 ml)

Product information

Clone: E17-L	Preservative: 0.05% NaN ₃
Buffer: 20 mM Tris-HCl, pH 8.0	Specificity: Human
Stabilizer: 20 mg/ml BSA	Storage: +4°C
Protein accession number: Q13112	Expiration: 24 months from the day of delivery

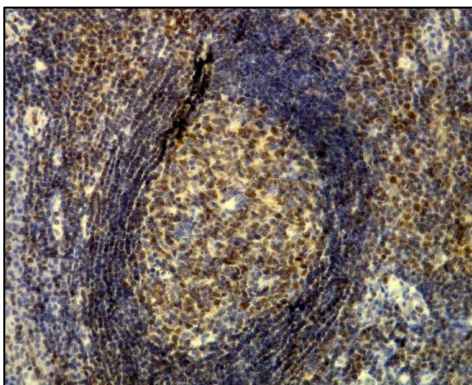
Immunogen: Peptide derived from the C-terminal region of human p60 sequence
Cellular localization: nucleus, cytoplasm
Positive control: colorectal adenocarcinoma tissue
Application: IHC-P **dilution 1:100 – 1:200**; IHC-Fr dilution to be tested by the user

Centrifuge the vial before use!

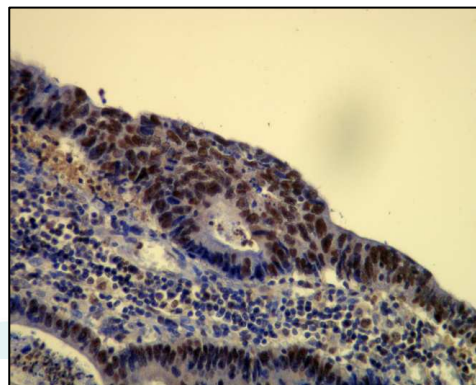
IHC-P protocol

1. Deparaffinize the section in 3 changes of xylene, 10 minutes each.
2. Wash the section in 96%, 80% and 70% ethyl alcohol for 10 minutes each.
3. Rinse in distilled water, 2 x 5 minutes.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H₂O₂) for 10 minutes.
5. Wash in distilled water, 2 x 5 minutes.
6. For antigen retrieval: Immerse the slide in citrate buffer, pH 6.0* and incubate at 95-97°C in water bath for 25 minutes. Remove the staining to room temperature and let the slide to cool (in citrate buffer, pH 6.0) for 15 minutes.
7. Rinse in distilled water, 2 x 5 minutes.
8. Wash in PBS (phosphate buffer saline, pH 7,0-7,5) supplemented with 0.05% of Tween-20 (*buffer A*), 2 x 5 minutes.
9. Incubate the section with primary antibody diluted in *PBS buffer (without Tween-20)* at the **dilution 1:100 – 1:200** for 1 hour in the closed wet chamber.
10. Wash 3 x 5 minutes with *buffer A*.
11. Apply the secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol (HRP - Peroxide - DAB). Micropolymer-HRP detection kit rabbit/mouse dual of DB Biotech is suggested (<http://www.dbbiotech.com/products/detection-system.html>)..
12. Wash 3 x 5 minutes with *buffer A*.
13. Apply the chromogen (DAB), 1-5 minutes.
14. Wash in water, 2 x 5 minutes.
15. Stain in hematoxylin for 5 minutes.
16. Wash in distilled water, 3 x 2 minutes.
17. Mount the slide for observation.

A



B



Nuclear expression of p60 protein in lymphocytes of the germinal centre of the lymph node **(A)** and colorectal adenocarcinoma **(B)**, detected with anti-p60 monospecific clonal antibody (DB203).

Kindly performed and provided by Marián Švajdler, MD and Dr. Lucia Fröhlichová, from Louis Pasteur University Hospital Department of Pathology, Košice, Slovak Republic.

*** Citrate Buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0):**

Citric acid (anhydrous) ----- 1.92 g; Distilled water ----- 1000 ml
 Mix to dissolve in 700 ml of distilled water. Adjust pH to 6.0 with 1M NaOH 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.