



Anti - CD117

Rabbit clonal antibody

CAT#

CONCENTRATED

READY TO USE (RTU)

DB 062-0.1 $(100 \mu l)$ DB 062-0.2 (200 µl)

DB 062-RTU-7 (7 ml) DB 062-RTU-15 (15 ml)

DB 062-0.5 (500 µl) DB 062-1 (1 ml)

STORAGE AND APPLICATION

CONCENTRATED

Storage: +4°C, Do not freeze!

Storage: Application: IHC-P.

dilution 1:100

READY TO USE (RTU)

Application: IHC-P. ready to use

PRODUCT INFORMATION

Clone: R21-V

20 mM Tris-HCl, pH 8.0 Buffer:

Stabilizer: 20 mg/ml BSA Preservative: 0.05% NaN₃

Specificity: Human

Expiration: 24 months from the shipping date

Peptide derived from C-terminal sequence of human Immunogen:

CD117. Antibody recognizes the epitope between Ile957

- His973.

Cellular localization: membrane, cytoplasm

Positive control: human large bowel stromal tumor tissue

Protein accession number: P10721

IHC-P PROTOCOL - INSTRUCTION MANUAL

- Deparaffinize the section in 3 changes of xylene, 5 minutes each.
- Wash the section in 96%, 80% and 70% ethyl alcohol for 10 minutes each.
- Rinse in distilled water.
- Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H₂O₂) for 10 minutes.
- Rinse in distilled water.
- For antigen retrieval: immerse the slide in the citrate buffer, pH 6.0, 0.05% Tween-20*, and incubate in water bath at 96-98°C for 30-40 minutes. (Alternatively adjust to your own protocol, keeping the required pH)
- Transfer the slide to room temperature and let it cool down (in citrate buffer, pH 6.0) for 15 minutes.
- Rinse in distilled water
- Wash in 0.05 M Tris-HCl, pH 7.6 buffer supplemented with 0.2% of Tween-20 (buffer A) for 5 minutes
- CONCENTRATED:

Incubate the section with primary antibody at the dilution 1:100 for 1 hour in the closed wet chamber.

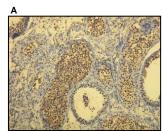
READY TO USE (RTU):

Incubate the section with primary antibody (ready to use) for 1 hour in a 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). Micropolymer-HRP detection kit rabbit/mouse dual of DB Biotech is suggested (http://www.dbbiotech.com/products/detection-system.html).
- Wash twice 5 minutes with buffer A.
- 14. Apply the chromogen (DAB), 1 3 minutes.
- Rinse in water.
- 16. Stain in hematoxylin for 5 minutes.
- 17. Wash in water 10 minutes.
- 18. Dehydrate the section in 2 changes of 96% ethyl alcohol for 5 minutes each.
- Wash the section in 2 changes of xylene for 2 minutes each.
- 20. Mount the slide for observation.

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

-- 1000 ml ----- 1.92 g; Distilled water Citric acid (anhydrous) ----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.





SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM

PROCEDURE: U ultraView DAB

- Deparafinization 1.
- Heating (72 °C) at the medium temperatures. Deparafinization. 2.

VENTANA PROTOCOL - INSTRUCTION MANUAL

- Cell conditioning 3.
- ULTRA conditioner #2
- Heating glass (95 °C), incubation 8 min. (Cell conditioner #2; buffer CC2). 5.
- ULTRA CC2 solution application 24 min. 6.
- Antibody incubation temperature
- 8. Heating glass (36 °C), incubation 4 min.
- 9. Titration
- 10. Hand apply - primary antibody 100 µl. Incubation 32 min. 11. ultraWash
- Nuclear stain
- Hematoxylin II application one drop (nuclear stain). Cover and incubate 12 min.
- After nuclear stain
- 15. Bluing reagent application, one drop. After nuclear stain, cover and incubate 4 min

LEICA BOND MAX PROTOCOL - INSTRUCTION MANUAL

SHORT APPLICATION PROTOCOL FOR LEICA BOND MAX SLIDE STAINING SYSTEM

Protocol F:

- Visualization system: BOND Refine DS9800
- Epitope retrieval / heating time / temperature: ER1 / 20 min. / 100 °C
- Incubation of primary antibody / temperature: 30 min. / 20 °C

PRECAUTIONS

- We strongly recommend to use DB Primary Antibody Diluent (catalog number DB D-125, or DB D-250), eventually alternative diluent (containing protease free BSA at the concentrations ≥ 1mg/ml) for dilution of concentrated antibodies, otherwise the warranty might be voided.
- Centrifuge the vial before use.
- Intended for professional In Vitro Diagnostic use in laboratories.
- Do not use after expiration date stamped on vial label.
- Avoid contamination of the reagent.
- Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
- The reagent contains sodium azide (NaN₃) which is highly toxic in higher concentrations. The concentration in the reagent (0.05%) is not considered as hazardous.
- Disposal of waste material must be conducted in accordance with local regulations.
- Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

Membranous CD117 positivity in the classical seminoma (A, B). All, formalin fixed,