Anti - C3d complement

Rabbit monoclonal antibody

STORAGE AND APPLICATION

CONCENTRATED	READY TO USE (RTU)
Storage: +4˚C	**Storage:** +4˚C, Do not freeze!
Application: IHC-P, dilution 1:100 - 1:200	**Application:** IHC-P, ready to use

PRODUCT INFORMATION

Clone:	E28-P
Buffer:	20 mM Tris-HCl, pH 8.0
Stabilizer:	20 mg/ml BSA
Preservative:	0.05% NaN_3
Specificity:	Human
Expiration:	24 months from the shipping date
Immunogen:	Peptide derived from N-terminal sequence of human C3d complement fragment.
Cellular localization:	secreted
Positive control:	human skin tissue
Protein accession number:	P01024

CENTRIFUGE THE VIAL BEFORE USE!

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 (H_2O_2) for 10 minutes.
5. Wash in distilled water, 2 x 5 minutes.
6. For antigen retrieval: immerse the slide in Tris-EDTA buffer, pH 9.0*, and incubate in water bath 40 min at 96-98˚C
7. Remove the staining to room temperature and let the slide to cool (in Tris-EDTA buffer, pH 9.0) for 20 minutes.
8. Rinse in distilled water, 2 x 5 minutes.
9. Wash in 0.05 M Tris-HCl, pH 7.6 buffer supplemented with 0.2% of Tween-20 or PBS buffer supplemented with 0.2% of Tween-20 (**buffer A**), 2 x 5 minutes
10. **CONCENTRATED:**
    Incubate the section with primary antibody at the **dilution 1:100 - 1:200** 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 3 x 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.

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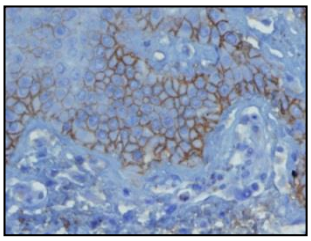
13. Wash 3 x 5 minutes with buffer A.
14. Apply the chromogen (DAB), 1 - 3 minutes.
15. Wash in water, 2 x 5 minutes.
16. Rinse in CuSO4.5H2O solution /0,90g NaCl + 0,50g CuSO4.5 H2O in 100ml distilled water/
17. Wash in distilled water, 1 x 2 minutes.
18. Stain in hematoxylin for 5 minutes.
19. Wash in distilled water, 3 x 2 minutes.
20. Rinse in ammonium hydroxide solution (37mM), 1 min.
21. Wash in distilled water, 1 x 2 minutes.
22. Mount the slide for observation.

*** Tris-EDTA Buffer (10mM Tris Base, 1mM EDTA solution, 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. Adjust the final volume to 1 liter with distilled water.
Store this solution at room temperature for 3 months or at +4ºC for longer storage.



Skin biopsy from the lesion of the early pemphigus vulgaris (without blister formation), stained with anti-C3d complement (DB 106) antibody shows strong positive intraepidermal intercellular immunostaining. Formalin fixed, paraffin embedded human tissue (4 µm section) stained according to related DB Biotech datasheet.

VENTANA PROTOCOL – INSTRUCTION MANUAL

SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM

1. Drying (Enter).
2. Heating glass (72˚C), incubation 4 min. Drying.
3. Deparafinization (Enter).
4. Heating (72˚C) with the medium temperatures. Deparafinization.
5. Prolonged deparafinization (Enter).
6. Cell conditioning (Enter).
7. ULTRA conditioner #1 (Enter).
8. Heating glass (97˚C), incubation 8 min (Cell conditioner #1).
9. ULTRA CC1 solution application – 20 min (Enter).
10. ULTRA CC1 solution application – 36 min (Enter).
11. ULTRA CC1 solution application – 52 min (Enter).
12. Titration (Enter).
13. Hand apply – primary antibody. Incubation 56 min.
14. Nuclear stain (Enter).
15. Hematoxylin application – one drop (nuclear stain). Cover and incubate 8 min.
16. After nuclear stain (Enter).
17. Bluing reagent application, one drop. After nuclear stain, cover and incubate 8 min.

PRECAUTIONS

1. Intended for professional In Vitro Diagnostic use in laboratories.
2. Do not use after expiration date stamped on vial label.
3. Avoid contamination of the reagent.
4. Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
5. The reagent contains sodium azide (NaN_3) which is highly toxic in higher concentrations. The concentration in the reagent (0.05%) is not considered as hazardous.
6. Disposal of waste material must be conducted in accordance with local regulations.
7. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.