Anti - C4d complement

Rabbit clonal antibody

CAT#

CONCENTRATED		READY TO USE (RTU)	
DB 107-0.1	(100 µl)	DB 107-RTU-7	(7 ml)
DB 107-0.2	(200 µl)	DB 107-RTU-15	(15 ml)
DB 107-0.5	(500 µl)		
DB 107-1	(1 ml)		

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: A24-T
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 internal sequence of human C4.

Cellular localization: secreted
Positive control: transplanted kidney tissue
Protein accession number: P0C0L4

IHC-P PROTOCOL – INSTRUCTION MANUAL

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 for 20-25 minutes 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 of DB Biotech is suggested (http://www.dbbiotech.com/products/detection-system.html).
13. Wash 3 x 5 minutes with buffer A.
14. Apply the chromogen (DAB), 1 - 3 minutes.
15. Wash in distilled water, 2 x 5 minutes.
16. Rinse in solution $CuSO_4 . 5H_2O$ /0.90g NaCl + 0.50g $CuSO_4.5H_2O$ in 100ml distelled 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 37mM amonium hydroxide solution.
21. Wash in distilled water, 1 x 2 minutes.
22. Mount the slide for observation.

*** 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 +4ºC for longer storage.

VENTANA PROTOCOL – INSTRUCTION MANUAL

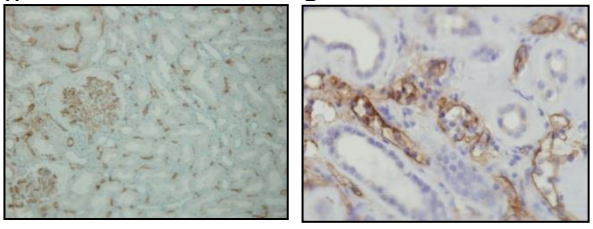
SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM

1. Drying (Enter).
2. Heating glass (72°C), incubation 4 min. Drying.
3. Deparafinization.
4. Heating (72°C) with the medium temperatures. Deparafinization.
5. Prolonged deparafinization (Enter).
6. Cell conditioning (Enter).
7. ULTRA conditioner #2 (Enter).
8. Heating glass (99°C), incubation 8 min (Cell conditioner #2).
9. ULTRA CC2 solution application – 24 min (Enter).
10. ULTRA CC2 solution application – 44 min (Enter).
11. Titration (Enter).
12. Hand apply – primary antibody. Incubation 56 min.
13. Nuclear stain (Enter).
14. Hematoxylin application – one drop (nuclear stain). Cover and incubate 8 min.
15. After nuclear stain (Enter).
16. Bluing reagent application, one drop. After nuclear stain, cover and incubate 8 min.

PRECAUTIONS

1. **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.**
2. **Centrifuge the vial before use.**
3. Intended for professional In Vitro Diagnostic use in laboratories.
4. Do not use after expiration date stamped on vial label.
5. Avoid contamination of the reagent.
6. Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
7. 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.
8. Disposal of waste material must be conducted in accordance with local regulations.
9. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

A **B**



Transplanted kidney tissue stained with anti-C4d complement (DB 107) antibody shows diffuse strong positive immunostaining of peritubular and glomerular capillaries **(A)**, indicating acute antibody mediated rejection and diffuse strong positive immunostaining of dilated peritubular moderate capillaries **(B)**, indicating acute antibody mediated rejection. Both, formalin fixed, paraffin embedded human tissues (4 µm sections) stained with anti - C4d complement (DB 107) monospecific clonal antibody according to related DB Biotech datasheet.

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Revision Date: 22.05.2017