Anti - Melan A

Rabbit clonal antibody

CAT#

CONCENTRATED		READY TO USE (RTU)	
DB 050-0.1	(100 µl)	DB 050-RTU-7	(7 ml)
DB 050-0.2	(200 µl)	DB 050-RTU-15	(15 ml)
DB 050-0.5	(500 µl)		
DB 050-1	(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: A19-P
Buffer: 20 mM Tris-HCl, pH 8.0
Stabilizer: 20 mg/ml BSA
Preservative: 0.05% NaN_3

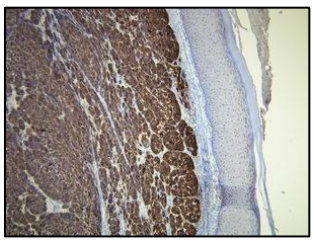
Specificity: Human
Expiration: 24 months from the shipping date
Immunogen: Peptide derived from C-terminal sequence of human Melan A. Antibody recognizes the epitope between Glu105 - Pro115.

Cellular localization: membrane,secreted
Positive control: human melanoma tissue
Protein accession number: Q16655

IHC-P PROTOCOL – INSTRUCTION MANUAL

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% ethyl alcohol for 10 minutes each.
3. Rinse in distilled water.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H_2O_2) for 10 minutes.
5. Wash in distilled water for 5 minutes.
6. For antigen retrieval: immerse the slide in the citrate buffer, pH 6.0, 0.05% Tween-20*, and incubate in water bath at 96-98ºC for 30-40 minutes. (Alternatively adjust to your own protocol, keeping the required pH)
7. Transfer the slide to room temperature and let it cool down (in citrate buffer, pH 6.0) 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. **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 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).
13. Wash twice 5 minutes with buffer A.
14. Apply the chromogen (DAB), 1 - 3 minutes.
15. Wash in water for 10 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water for 10 minutes.
18. Dehydrate the section in 2 changes of 96% ethyl alcohol for 5 minutes each.
19. 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):**
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.



Diffuse Melan A positivity in the cutaneous malignant melanoma. Formalin fixed, paraffin embedded human tissue (4 µm section) stained with anti - Melan A (DB 050) monospecific clonal antibody according to related DB Biotech datasheet.

VENTANA PROTOCOL – INSTRUCTION MANUAL

SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK ULTRA IHC/ISH STAINING MODULE

1. Deparafinization (Enter).
2. Heating (74°C) at the medium temperatures. Deparafinization.
3. Extended deparafinization. (Enter)
4. Cell conditioning (Enter).
5. ULTRA Conditioner #1 (Enter).
6. Heating glass (95°C), incubation 8 min (Cell conditioner #1).
7. ULTRA CC1 solution application – 20 min. (Enter).
8. ULTRA CC1 solution application – 36 min. (Enter).
9. ULTRA CC1 solution application – 52 min. (Enter).
10. ULTRA CC1 solution application – 64 min. (Enter).
11. Temperatures for antibody incubation (Enter)
12. Slides warm up to 36°C and antibody incubation for 4. min. (Antibody)
13. Titration (Enter).
14. Hand apply – primary antibody. Incubation 32 min.
15. Nuclear stain (Enter).
16. Hematoxylin II application – one drop (nuclear stain). Cover and incubate 8 min.
17. After nuclear stain (Enter).
18. 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.

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