# Anti – Melanosome

## *Rabbit clonal antibody*

### CAT#

| CONCENTRATED | | READY TO USE (RTU) | |
|---|---|---|---|
| DB 049-0.1 | (100 µl) | DB 049-RTU-7 | (7 ml) |
| DB 049-0.2 | (200 µl) | DB 049-RTU-15 | (15 ml) |
| DB 049-0.5 | (500 µl) | | |
| DB 049-1 | (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 | **Application:** IHC-P, ready to use |

### PRODUCT INFORMATION

**Clone:** P14-V
**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 Melanosome/gp100. Antibody recognizes the epitope between Ile649 - Val661.

**Cellular localization:** membrane, secreted
**Positive control:** human melanoma tissue

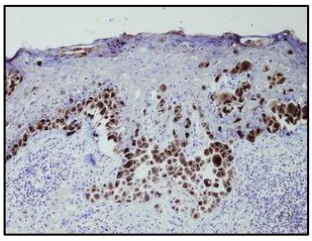
**Protein accession number:** P40967

### 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** 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.

**Direct application of primary antibody in buffer A without previous antigen retrieval is optional.



Diffuse anti Melanosome antibody positivity in the cutaneous malignant melanoma. Formalin fixed, paraffin embedded human tissue (4 µm section) stained with anti - Melanosome (DB 049) monospecific clonal antibody according to related DB Biotech datasheet.

### VENTANA PROTOCOL – INSTRUCTION MANUAL

**SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM**

**PROCEDURE: U ultraView DAB**

1. Deparafinization
2. Heating (72 °C) at the medium temperatures. Deparafinization.
3. Cell conditioning
4. ULTRA conditioner #2
5. Heating glass (95 °C), incubation 8 min. (Cell conditioner #2; buffer CC2).
6. **ULTRA CC2** solution application – **44 min.**
7. Antibody incubation temperature
8. Heating glass (36 °C), incubation 4 min.
9. Titration
10. Hand apply – primary antibody 100 µl. Incubation **36 min.**
11. ultraWash
12. Nuclear stain
13. Hematoxylin II application – one drop (nuclear stain). Cover and incubate 12 min.
14. 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 / 30 min. / 100 °C**
- Incubation of primary antibody / temperature: **30 min. / 20 °C**

### 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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