

## Anti - C-erb-2 (Her-2/neu) – rabbit clonal antibody

Cat. #: DB 094-0.1 (100 µl)      DB 094-1 (1 ml)  
DB 094-0.5 (500 µl)      DB 094-T (test sample)

### Product information

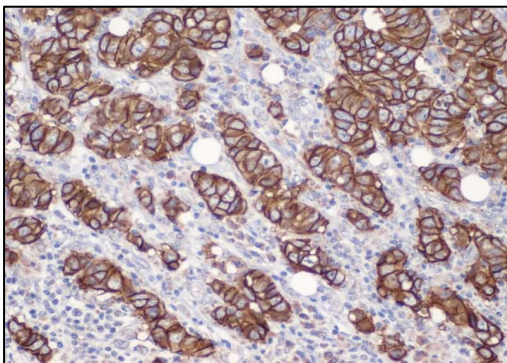
<b>Clone:</b> A24-V	<b>Preservative:</b> 0.05% NaN <sub>3</sub>
<b>Buffer:</b> 20 mM Tris-HCl, pH 8.0	<b>Specificity:</b> Human
<b>Stabilizer:</b> 20 mg/ml BSA	<b>Storage:</b> -20°C
<b>Protein accession number:</b> P04626	<b>Expiration:</b> 18 months from the day of delivery

**Immunogen:** Peptide derived from C-terminal region of human C-erb-2  
**Application:** IHC-P dilution 1:100 - 300; IHC-fr dilution to be tested by the user

**Centrifuge the vial before use**

### IHC-P Protocol

1. Deparaffinize the section in 3 changes of xylene, 5 minutes each.
2. Wash the section in 96%, 80% and 70% benzyl alcohol for 5 minutes each.
3. Rinse in distilled water.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 10 minutes.
5. Wash in distilled water.
6. For antigen retrieval use one of the following procedures: **A)** Immerse the slide in Tris-EDTA buffer, pH 9.0, 0.05% Tween- 20\*, and incubate in microwave (600W) for 10 minutes, or **B)** Immerse the slide in citrate buffer, pH 6.0, 0.05% Tween-20\*\*, and incubate in microwave (600W) for 10 minutes. (*Alternatively adjust to your own protocol, keeping the required pH*)
7. Remove the staining to room temperature and let the slide to cool for 15 minutes.
8. Rinse in distilled water.
9. Wash in 0.05 M Tris-HCl , pH 7.6 buffer supplemented with 0.5% of Tween-20 (*buffer A*) for 5 minutes.
10. Incubate the section with primary antibody diluted in *buffer A* at the **dilution 1:100 - 300** for 1 hour in the 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).
13. Wash twice 5 minutes with *buffer A*.
14. Apply of chromogen (DAB), 10 minutes.
15. Wash in water – 10 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water – 10 minutes.
18. Dehydrate the section in 2 changes of 96% benzyl alcohol for 5 minutes each.
19. Wash the section in 2 changes of xylene for 2 minutes each.
20. Mount the slide for observation.



Formalin-fixed and paraffin-embedded human breast ductal carcinoma tissue (4 µm) stained with anti-C-erb-2 antibody (DB 094) shows strong membranous immunostaining of neoplastic cells.

Kindly performed and provided by Katarína Poliaková, MD and Ľubomír Straka, MD, Ph. D. from Clinical Pathology Prešov, Ltd., Prešov, Slovak republic.

**\* 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 +4°C for longer storage.

**\*\* 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.