Anti - iNOS

Rabbit clonal antibody

CAT#

CONCENTRATED		READY TO USE (RTU)	
DB 003-IHC-0.1	(100 µl)	DB 003-IHC-RTU-7	(7 ml)
DB 003-IHC-0.2	(200 µl)	DB 003-IHC-RTU-15	(15 ml)
DB 003-IHC-0.5	(500 µl)		
DB 003-IHC-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: K13-A
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 human iNOS sequence. Antibody recognizes the epitope between Ser1118 - Gly1129.

Cellular localization: cytoplasm, membrane
Positive control: liver, lung tissue

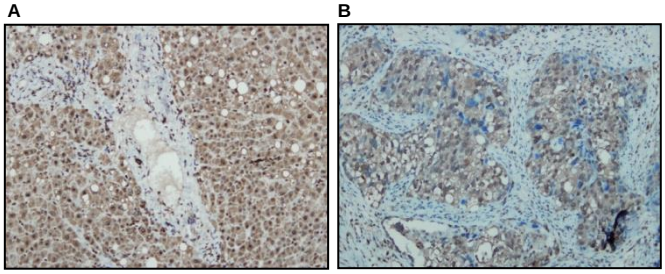
Protein accession number: P35228

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.
6. For antigen retrieval: Immerse the slide in Tris-EDTA buffer*, pH 9.0 and incubate at 95-97ºC in water bath for 25 minutes.
7. Remove the staining to room temperature and let the slide to cool (in Tris-EDTA buffer, pH 9.0) for 15 minutes.
8. Rinse in distilled water, 2 x 5 minutes.
9. Wash in PBS (phosphate buffer saline, pH 7.0-7.5) supplemented with 0.05% of Tween-20 (**buffer A**), 2 x 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 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 water, 2 x 5 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in distilled water, 3 x 2 minutes.
18. 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 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.

A **B**



Liver tissue **(A)** and lung adenocarcinoma tissue **(B)** stained with anti-iNOS (DB 003-IHC) antibody shows strong positive immunostaining of hepatocytes and lung cancer cells. Formalin fixed, paraffin embedded human tissues (4 µm sections) stained 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 #1
5. Heating glass (95 °C), incubation 8 min. (Cell conditioner #1; buffer CC1).
6. **ULTRA CC1** solution application – **20 min.**
7. Antibody incubation temperature
8. Heating glass (36 °C), incubation 4 min.
9. Titration
10. Hand apply – primary antibody 100 µl. Incubation **52 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: **ER2 / 20 min. / 100 °C**
- Incubation of primary antibody / temperature: **30 min. / 20 LT**

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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