Anti – p16 – *rabbit clonal antibody*

CAT#

CONCENTRATED		READY TO USE (RTU)	
DB 152-0.1	(100 µl)	DB 152-RTU-7	(7 ml)
DB 152-0.2	(200 µl)	DB 152-RTU-15	(15 ml)
DB 152-0.5	(500 µl)		
DB 152-1	(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: R19-D
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 day of delivery
Immunogen: Peptide derived from C-terminal sequence of human p16.

Cellular localization: cytoplasm, nucleus
Positive control: cervical carcinoma tissue

Protein accession number: P42771

CENTRIFUGE THE VIAL BEFORE USE!

IHC-P PROTOCOL

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 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 diluted in PBS buffer (without Tween-20) 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 +4°C for longer storage.

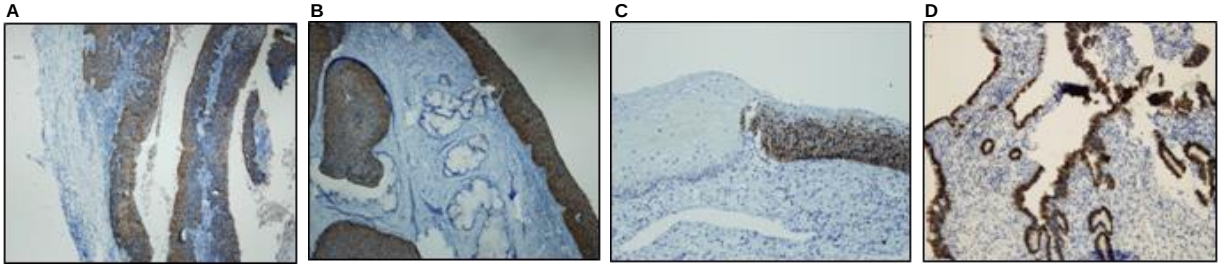
VENTANA PROTOCOL

SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM

1. Drying (Enter).
2. Heating (72°C), incubation 4 min; drying.
3. Deparafinization (Enter).
4. Heating (72°C) with the medium temperatures.
5. Cell conditioning (Enter).
6. ULTRA conditioner #1 (Enter).
7. Heating glass (95°C), incubation 8 min (Cell conditioner #1).
8. ULTRA CC1 solution application – 20 min (Enter).
9. ULTRA CC1 solution application – 36 min (Enter).
10. Titration (Enter).
11. Hand Apply – primary antibody. Incubation 36 min.
12. Nuclear stain (Enter).
13. Hematoxylin application – one drop (Nuclear stain). Cover and incubate 4 min.
14. After nuclear stain (Enter).
15. Bluing reagent application, one drop. After nuclear stain, cover and incubate 4 min.

PRECAUTIONS

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



Formalin-fixed and paraffin-embedded human tissues (4 µm) corresponding to: **A)** Cervical lymph node metastasis of oropharyngeal HPV-associated squamous cell carcinoma; **B)** High grade squamous intraepithelial lesion of the uterine cervix, in contrast with normal endocervical glands; **C)** High grade squamous intraepithelial lesion of the uterine cervix, in contrast with non-dysplastic metaplastic squamous epithelium; **D)** Adenocarcinoma in situ of the uterine cervix, stained with anti-p16 (DB 152), show strong and specific positive immunostaining of dysplastic and neoplastic epithelium, with no reactivity in normal epithelial and stromal structures.
Kindly performed and provided by Marián Švajdler, MD and Dr. Lucia Fröhlichová, from Louis Pasteur University Hospital Department of Pathology, Košice, Slovak republic.

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