Anti - Ki-67

Rabbit clonal antibody

CAT#

CONCENTRATED		READY TO USE (RTU)	
DB 111-0.1	(100 µl)	DB 111-RTU-7	(7 ml)
DB 111-0.2	(200 µl)	DB 111-RTU-15	(15 ml)
DB 111-0.5	(500 µl)		
DB 111-1	(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: E18-E
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 internal region of human Ki-67. Antibody recognizes the epitope between Asp1101 - Glu1116.

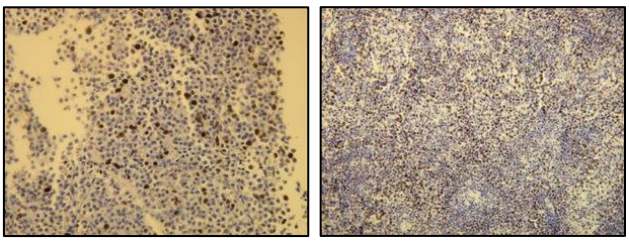
Cellular localization: nucleus
Positive control: tonsil tissue
Protein accession number: P46013

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, 0.05% Tween 20*, and incubate in water bath at 95˚C for 30 or 60 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.
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 – 10 minutes.
16. Stain in hematoxylin for 5 minutes.
17. Wash in water – 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.

***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.

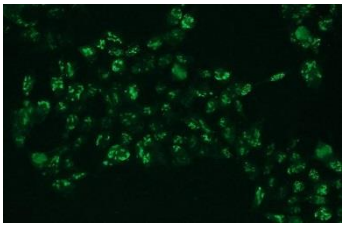
A **B**



Proliferating cells of the plasma cell myeloma in the bone marrow biopsy **(A)** and B-cell lymphoma showing high proliferating index **(B)** visualised by the Ki-67 immunohistochemistry. Both, formalin fixed, paraffin embedded human tissues (4 µm sections) stained with anti - Ki-67 (DB 111) monospecific clonal antibody according to related DB Biotech datasheet.

ICC PROTOKOL – INSTRUCTION MANUAL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let air-dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS.
2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT.
3. Wash 2 x 3 min with PBS.
4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice.
5. Wash 2 x 3 min with PBS.
6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT.
7. Incubate the cells with primary antibody: anti-Ki67 clonal antibody at the dilution of **1:100 - 1:200** in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber.
8. Wash 2 x 3 min with PBS.
9. Apply the secondary antibody (*in this case, the goat anti-rabbit IgG-FITC from Jackson Immunoresearch, cat. # 111-095-00, was used* at 1:300 in antibody dilution buffer, and cells were incubated for 1 hour at RT in dark).
10. Wash 3 x 3 min with PBS.
11. Rinse once with distilled water.
12. Mount the slide for observation, with a drop of anti-fade mounting medium.



Representative picture of Ki-67 expression in HEK293 cells, visualized with clonal rabbit anti-Ki-67 monospecific antibody. Primary antibody dilution - 1:100.

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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