p16-INK4A monoclonal antibody

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Product Details:

Size: 100 µg Species Reactivity: P16-INK4A human Published Species: N/A Host/Isotype: Mouse / IgG1 Class: Monoclonal Type: Antibody Clone: 12B1 Conjugate: Unconjugated Immunogen: p16^{INK4A} human Form: Liquid Concentration: 0.1 mg/mL Purification: Protein A/G Storage buffer: PBS, pH 7.2 Contains: 0.1% Sodium Azide Storage conditions: 4°C

Applications Tested Dilution

Western Blot (WB) 1:10 - 1:50 Immunohistochemistry (IHC) 1:5-1:10 Immunohistochemistry (Paraffin) (IHC (P)) 1:100 Immunohistochemistry (PFA fixed) (IHC (PFA)) 1:100 Immunocytochemistry (ICC/IF) 1:100 ELISA (ELISA) 1:20 -1:200

Recommended dilution:

Application	Dilution
Western Blot (WB)	WB:1:10-1:100
Immunoprecipitation (IP)	IP : 1 - 4.0 μg for 1.0-2.0 mg of total protein lysate
Immunohistochemistry (IHC)	IHC :1:5-1:10
Flow Cytometry (FC)	FC : 1 μg per 10 ⁶ cells in a 100 μl suspension



Deparaffinize and rehydrate

1. Deparaffinize slides in 2 baths of xylene for 10 min each.

2. Rehydrate slides by sequential incubation with 100%, 95%, 80%, and 60% ethanol, 5 min for each bath and immerse slides with distilled water 3 times for 1 min each.

Antigen Retrieval

1. Transfer slides to a container and cover with antigen retrieval solution

and heat slides in a microwave on medium power for 5 min.

3. Allow slides to cool in the buffer for 10 min.

Incubation with Primary Antibody and Signal Detection

1. Rinse slides 3 times with 1X TBS for 1 min each.

2. Incubate slides with 3% H2O2 solution (diluted in distilled water) for 10 min.

- 3. Rinse slides 3 times with 1XTBS for 1 min each.
- 4. Block the slides at room temperature for 1 h in Blocking buffer.
- 5. Incubate slides with primary antibody for 1h at room temperature.

6. Rinse slides 3 times with 1X TBST for 1 min each.

7. Incubate slides with sufficient peroxidase labeled secondary antibody for 30 min at room temperature.

8. Rinse slides 3 times with 1X TBS for 1 min each.

9. Incubate slides with enough DAB for 5 min at room temperature or until a brown color develops.

10. Rinse slides 3 times with distilled water for 1 min each.

11. Incubate slides in a bath of hematoxylin at room temperature to stain the nuclei for 3 min.

12. Rinse slides 3 times with 1X TBS for 1 min each.

13. Immerse slides in distilled water for 5 min.

14. Immerse slides sequentially into 60%, 80%, 90%, and 100% ethanol bath for 5 min each.

15. Immerse slides in xylene bath for 5 min, then immerse in another fresh xylene for 5 min.

16. Mount the slides with a small drop of neutral balsam and add a coverslip. Air-dry slides in the hood.

17. Examine slides under a microscope.

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IHC Protocol:

Clone 12B1 1:2 dilution Clone 12B1 1:10 dilution





12B1 mAb



Objectives 5x and 20x





Western blot analysis of extracts from HeLa cells using p16^{INK4A} (12B1) mouse monoclonal antibody clone at 1:100 dilution

