

# Instruction for Use

### [Product name]

MDM2 Antibody (Immunohistochemistry)

### [ Packing specification ]

0.2ml, 1ml, 3ml, 6ml, 11ml

### Intended use

This reagent is used for immunohistochemical staining based on routine staining to provide auxiliary information for physicians in diagnosis.

## [Principle]

MDM2 is a 90kD ubiquitin ligase for p53 and plays a central role in regulation of the stability of p53. MDM2 binds and inhibits transactivation role played by p53 and overexpression of MDM2 can result in the inactivation of p53 and decrease its tumor suppressor function. MDM2 also acts to stimulate cell proliferation via its induction of transcription factors such as E2F1 and DPI. In addition to p53, MDM2 is involved in processes of cell cycle, apoptosis, and tumorigenesis through interactions with proteins that include retinoblastoma 1 and ribosomal protein L5. Further supporting the role of MDM2 as an oncogene, several human tumor types have been shown to have increased levels of MDM2, including soft tissue sarcomas and osteosarcomas as well as breast tumors. Add the primary antibody to bind the antigen on tissue sections, and then use HRP labeled secondary antibody binding primary antibody to form the secondary antibody-primary antibody-antigen complex. When DAB chromogenic solution is added, HRP reacts with enzyme substrate to produce brown insoluble reaction product, which indirectly indicating the existence of antigen.

## [Main components]

Immunoglobulin, antibody diluent

## **Storage**

Store at  $2\sim8$ °C for 18 months.

# [Sample requirements]

FFPE tissues are usually cut into sections as thin as  $3\sim5\mu m$  with a microtome. These sections are then mounted onto glass slides that are coated with a tissue adhesive.

## [Protocol]

- 1. Sample preparation: Deparaffinize the slides in xylene I, II, III for 5 minutes separately; Transfer the slides once through 100%, 100%, 95%, 75% alcohols for 2 minutes respectively. Rinse slides with deionized water for 30 seconds.
- 2. Blocking: Block endogenous peroxidase activity by incubating sections in 3% H<sub>2</sub>O<sub>2</sub> solution at room temperature for 5 minutes. Rinse the slides with deionized water for 30 seconds.
- 3. Antigen retrieval: Heat the EDTA Antigen retrieval buffer to  $100^{\circ}$ C. Then place the slides in the boiled buffer for  $15^{\sim}20$  min. Naturally cool down for 30 minutes at room temperature (time can be extended if needed). Rinse the slides with washing buffer.
- 4. Soak slides in the washing buffer for 2 times, 3 minutes for each time. Shake off washing buffer from slides, and remove residual washing buffer around the tissue by absorbent paper, then use PAP pen to circle the tissue.
- 5. Primary antibody incubation: Add primary antibody to tissue, incubate at room temperature for 30 minutes. Rinse the slides in washing buffer for 3 times, 3 minutes for each time. If the antibody is concentrated, please dilute it to RTU(ready to use) according to the information on packing.
- 6. Secondary antibody: Drain the slides. Add secondary antibody to tissue and incubate at room temperature for 20 minutes. Rinse the slides in washing buffer for 3 times, 3 minutes for each time.
- 7. DAB: Drain the slides. Add DAB to the tissue and incubate at room temperature for 5 min. Rinse slides with deionized water
- 8. Hematoxylin staining: Drain the slides. Add Hematoxylin to the tissue and incubate at room temperature for 5 minutes. Rinse slides with water. Use the acid solution for differentiation. Rinse slides with water.
- 9. Dehydrate: Dehydrate the slides in 75%, 95%, 100% alcohols for 2 minutes separately. Dry the slides and cover them with coverslips by mounting medium.

### **[** Positive localization **]**

- 1. Positive localization: nuclear.
- 2. Positive control: liposarcoma.

## [Precautions]

- 1. Please read the instruction carefully and become familiar with all components of the kit prior to use, Strictly follow the instruction during operation.
- 2. DO NOT use the kit or any kit component after their.
- 3. Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- 4. Avoid contact of skin, eyes and mucous membranes with the chemicals.
- 5. DO NOT pipet by mouth.
- 6. Unused reagents, used kit and waste must be disposed according to local regulations.



# 【Labels, Packing Logo Design】

| Symbol      | Introductions                                    | Symbol         | Introductions  |
|-------------|--|----------------|--|
| LOT         | Batch Code                                       | REF            | Catalogue number                                     |
| $\triangle$ | Warnings and Precautions                         | NON<br>STERILE | non-sterile  |
| IVD         | In Vitro diagnostic Medical device               | ~~ <u>~</u>    | Manufacture Date                                     |
|             | Manufacturer Name Address                        | EU REP         | Name and Address of European<br>Union Representative |
| (€          | CE Symbol  | UDI            | Unique Device Identification                         |
|             | Country of Manufacture                           | #              | Model Number   |
|             | Importer   |                | Distributor  |
| 2           | Do not reuse" are "single use,<br>"Use only once |                |  |

# [Manufacturer]



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