www.bioss.com.cn
sales@bioss.com.cn
techsupport@bioss.com.cn
400-901-9800

Human Phospho-MDM2 (Ser166) Ready-To-Use IHC Kit

Cat.No: IHC0548H
Applications: **IHC-P**
Reactivity: Human
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Human Phospho-MDM2 (Ser166) Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Human colon cancer)	1 slide	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Human Phospho-MDM2 (Ser166) Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

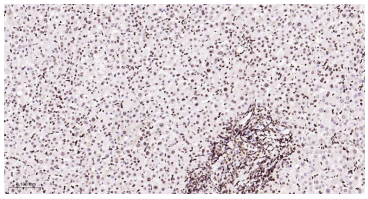
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0548H, Bioss Antibodies". Citation example: " Human Tissue sections using MDM2 IHC Kit (IHC0548H, Bioss Antibodies) were stained for MDM2 according to the manufacturer's instructions."

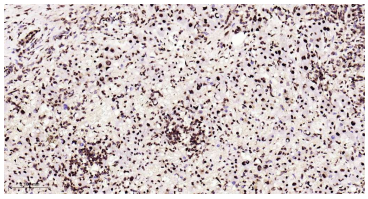
Introduction:

MDM2 is a ubiquitin ligase for p53 and plays a central role in regulation of the stability of p53. MDM2 is located on chromosome 12 on the q arm. Akt-mediated phosphorylation of MDM2 at Ser166 and Ser186 increases its interaction with p300, allowing MDM2-mediated ubiquitination and degradation of p53. Phosphorylation of MDM2 also blocks its binding to p19ARF, increasing the degradation of p53. MDM2 has also been shown to negatively regulate p53 function. 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. Another process by which MDM2 can inactivate p53 is by degrading p53 as the protein also possesses E3 ubiquitin ligase activity. Further, MDM2 plays important roles in apoptosis and cell cycle. MDM2 is over expressed in a wide range of human malignancies including soft tissue carcinomas and breast cancer. 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. More than 40 different alternatively spliced transcript variants of MDM2 have been isolated from both tumor and normal tissues.

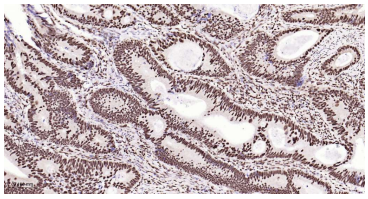
Validation Data



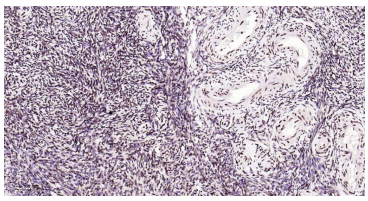
Immunohistochemical analysis of paraffin embedded Human liver tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).



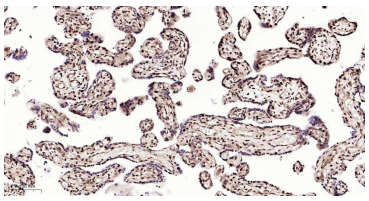
Immunohistochemical analysis of paraffin embedded Human hepatocellular carcinoma tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).



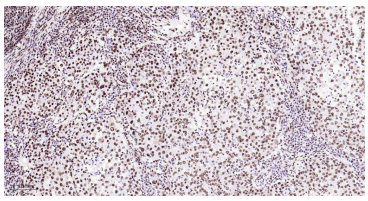
Immunohistochemical analysis of paraffin embedded Human colon cancer tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).



Immunohistochemical analysis of paraffin embedded Human ovary tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).



Immunohistochemical analysis of paraffin embedded Human placenta tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).



Immunohistochemical analysis of paraffin embedded Human gastric cancer tissue slide using IHC0548H (Human Phospho-MDM2 (Ser166) Kit).