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HMGB1 Ready-To-Use IHC Kit

Cat.No: IHC0566
Applications: **IHC-P**
Reactivity: Human, Mouse, Rat
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 (HMGB1 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, rat kidney, mouse kidney)	3 slides	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 **HMGB1 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.

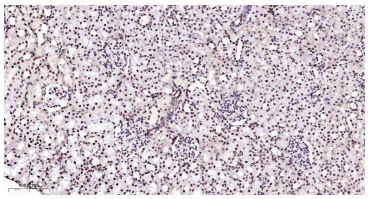
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0566, Bioss Antibodies". Citation example: " Tissue sections using HMGB1 IHC Kit (IHC0566, Bioss Antibodies) were stained for HMGB1 according to the manufacturer's instructions."

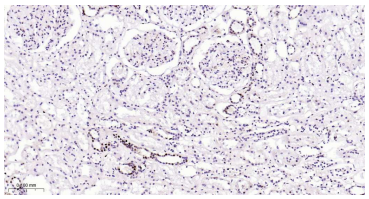
Introduction:

HMGB1 (High-mobility group box-1) protein was originally described as a nuclear non-histone DNA binding chromosomal protein. However, recent studies indicate that damaged, necrotic cells liberate HMGB1 into the extracellular milieu where it functions as a proinflammatory cytokine. Mouse HMGB1 is expressed as a 215 amino acid single chain polypeptide containing three domains: two tandem-linked positively charged DNA-binding domains (HMG box A, aa 9-79; and box B, aa 89-162), and a negatively charged 30 aa C-terminal acidic tail region. Residues 28 - 44 and 180 - 185 contain a nuclear localization signal (NLS). The cytokine activity of HMGB1 is contained in the B box, while the A box is associated with the helix-loop-helix domain of transcription factors. HMGB1 acts both as an inflammatory mediator that promotes monocyte migration and cytokine secretion, as well as a mediator of T cell-dendritic cell interaction. HMGB1 is secreted and acts to transduce cellular signals through its high affinity receptor, RAGE and possibly, TLR2 and TLR4. HMGB1 is highly conserved and ubiquitous in the nuclei and cytoplasm of nearly all cell types, is a necessary and sufficient mediator of inflammation during sterile and infection-associated responses. HMGB1 also act as DNA nuclear binding protein that has recently been shown to be an early trigger of sterile inflammation in animal models of trauma-hemorrhage via the activation of the Toll-like receptor 4 (TLR4) and the receptor for the advanced glycation endproducts (RAGE). Moreover, HMGB1 is reported that the level of HMGB1 is elevated during sterile tissue injury, infection, lethal endotoxemia or sepsis, collagen-induced arthritis, and ischemia-reperfusion induced tissue injury.

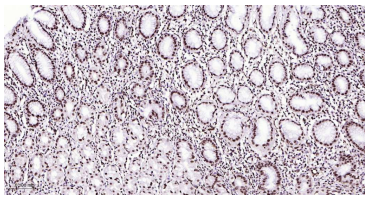
Validation Data



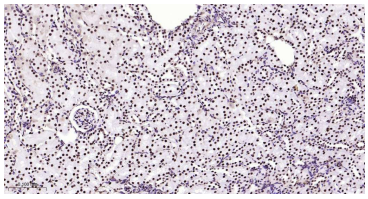
Immunohistochemical analysis of paraffin embedded Rat kidney tissue slide using IHC0566 (HMGB1 Kit).



Immunohistochemical analysis of paraffin embedded Human kidney tissue slide using IHC0566 (HMGB1 Kit).



Immunohistochemical analysis of paraffin embedded Human stomach tissue slide using IHC0566 (HMGB1 Kit).



Immunohistochemical analysis of paraffin embedded Mouse kidney tissue slide using IHC0566 (HMGB1 Kit).