

## Rat Ubiquitin Ready-To-Use IHC Kit

Cat.No: IHC0350R  
Applications: IHC-P  
Reactivity: Rat  
Size: 50T  
Assay type: Immunohistochemistry  
Sample type: FFPE tissue  
General Information:

| Number | Component   | Size    | Concentration | Storage                   |
|--------|---|---------|---------------|---------------------------|
| 1      | PBS Buffer (powder)   | 2 L X 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 (Rat Ubiquitin 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 (Rat colon)                                     | 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 **Rat Ubiquitin 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.

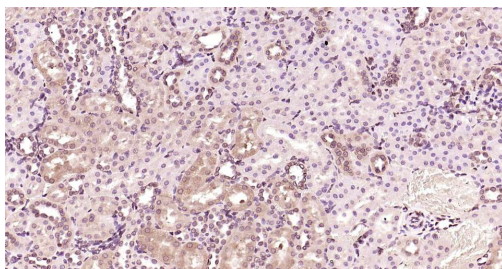
***Please cite this product as "IHC0350R, Bioss Antibodies". Citation example: "Rat Tissue sections using UBB IHC Kit (IHC0350R, Bioss Antibodies) were stained for UBB according to the manufacturer's instructions."***

## Introduction:

Ubiquitin, a highly conserved protein that has a major role in targeting cellular proteins for degradation by the 26S proteasome, is synthesized as a precursor protein consisting of either polyubiquitin chains or a single ubiquitin fused to an unrelated protein. This gene encodes a fusion protein consisting of ubiquitin at the N terminus and ribosomal protein S27a at the C terminus. When expressed in yeast, the protein is post-translationally processed, generating free ubiquitin monomer and ribosomal protein S27a. Ribosomal protein S27a is a component of the 40S subunit of the ribosome and belongs to the S27AE family of ribosomal proteins. It contains C4-type zinc finger domains and is located in the cytoplasm. Pseudogenes derived from this gene are present in the genome. As with ribosomal protein S27a, ribosomal protein L40 is also synthesized as a fusion protein with ubiquitin; similarly, ribosomal protein S30 is synthesized as a fusion protein with the ubiquitin-like protein fubi. Multiple alternatively spliced transcript variants that encode the same proteins have been identified.

## Validation Data

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Immunohistochemical analysis of paraffin embedded rat kidney tissue slide using IHC0350R (Rat Ubiquitin Kit).