

## Rat CD20 Ready-To-Use IHC Kit

**Cat. No.:** IHC0125R

**Sample Type:** FFPE tissue

**Size:** 50T (including 1 control slide)

**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.

### 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
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Rat CD20 Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Rat spleen)	1 slide	RTU	RT
13	Datasheet	1 copy		

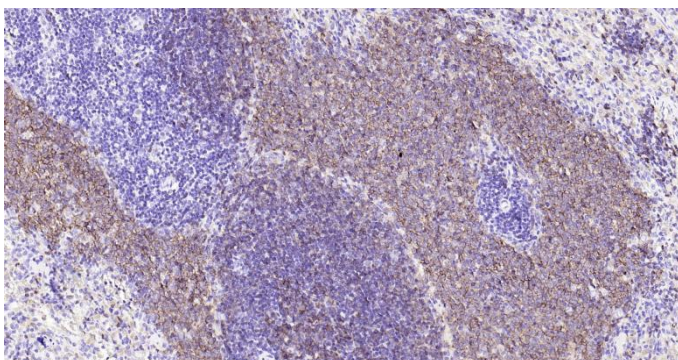
### Background

CD20 is a non-glycosylated surface phosphoprotein that has a molecular weight range of 33-37 kDa depending on the degree of phosphorylation. CD20 is expressed on mature and most malignant B cells, in a subpopulation of T lymphocytes and follicular dendritic cells. CD20 expression on B cells is synchronous with the expression of surface IgM and it regulates transmembrane calcium conductance, cell cycle progression and B-cell proliferation. CD20 is also associated with lipid rafts, but the intensity of this association depends on extracellular triggering, employing CD20 conformational change, and/or BCR (B cell antigen receptor) aggregation. After the receptor ligation, BCR and CD20 colocalize and then rapidly dissociate before BCR endocytosis, whereas CD20 remains at the cell surface. CD20 serves as a useful target for antibody-mediated therapeutic depletion of B cells, as it is expressed at high levels on most B-cell malignancies, but does not become internalized or shed from the plasma membrane following monoclonal antibody treatment. Diseases associated with CD20 dysfunction include Ms4a1-related common variable immune deficiency.

### Synonyms

CD20\_HUMAN; B-lymphocyte antigen CD20; B-lymphocyte surface antigen B1; Bp35; Leukocyte surface antigen Leu-16; Membrane-spanning 4-domains subfamily A member 1; MS4A1; MS4A2; B1; CVID5; LEU-16; S7.

## Validation Data



Immunohistochemical analysis of paraffin embedded Rat spleen tissue slide using IHC0125R (Rat CD20 IHC Kit).

## 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 CD20 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 **HRP-Goat anti-Rabbit IgG pAb** 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 " IHC0125R, Bioss Antibodies". Citation example: "Rat tissue sections using Rat CD20 IHC Kit (IHC0125R, Bioss Antibodies) were stained for CD20 according to the manufacturer's instructions."**