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

Rat CD44 Ready-To-Use IHC Kit

Cat.No: IHC0353R
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×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 CD44 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 thymus)	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 CD44 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.

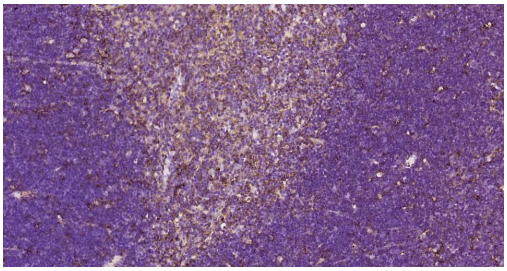
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0353R, Bioss Antibodies". Citation example: " Rat Tissue sections using CD44 IHC Kit (IHC0353R, Bioss Antibodies) were stained for CD44 according to the manufacturer's instructions."

Introduction:

CD44 cell surface antigen is a 100 kDa type 1 transmembrane glycoprotein widely expressed on human leucocytes, white matter of the brain and by some epithelial cells of the intestine and breast. Several isoforms of CD44 exist, including the predominant CD44H isoform detected in many normal tissues. CD44 is a receptor for hyaluronic acid (HA) and is involved in cell-cell interactions, cell adhesion and migration. CD44 also participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing. CD44 expression may be up-regulated upon some carcinomas, and it has been speculated that this may be related to metastatic potential. CD44 is expressed by hematopoietic, non-hematopoietic cells, epithelial tissues, and to filopodia in cultured keratinocytes. Further, bone marrow myeloid cells and memory T cells express CD44 at high levels, and peripheral B and T cells can upregulate the expression of CD44 in response to certain stimulatory events. Transcripts for the CD44 gene undergo complex alternative splicing that results in many functionally distinct isoforms, however, the full-length nature of some of these variants have not been determined. Alternative splicing is the basis for the structural and functional diversity of the CD44 protein. Diseases associated with CD44 dysfunction include superficial keratitis and lichen sclerosus. CD44 also may be related to tumor metastasis formation.

Validation Data



Immunohistochemical analysis of paraffin embedded rat thymus tissue slide using IHC0353R (Rat CD44 Kit).