www.biossusa.com
800.501.7654 [DOMESTIC]
+1.781.569.5821 [INT'L]
support@biossusa.com

Human HLA-DR Ready-To-Use IHC Kit

Cat. No.: IHC0193H
Sample Type: FFPE tissue **Size: 50T**
(including a 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	50T	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Human HLA-DR Recombinant Rabbit mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L / HRP)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
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 (Human spleen)	1 slide	RTU	RT
13	Datasheet	1 copy		

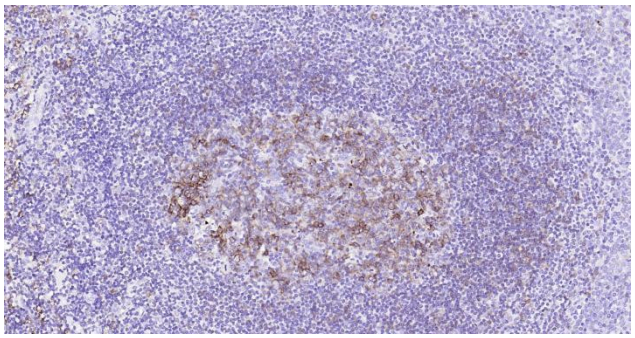
Background

HLA-DR, like other MHC class II molecules, is a transmembrane glycoprotein composed of a 36 kDa alpha chain (DRA) and 27 kDa beta chain (DRB). The alpha chain gene contains 5 exons. Exon 1 encodes the leader peptide, exons 2 and 3 encode the two extracellular domains, and exon 4 encodes the transmembrane domain and the cytoplasmic tail. DRA does not have polymorphisms in the peptide binding part and acts as the sole alpha chain for DRB1, DRB3, DRB4 and DRB5. Within the DR molecule the beta chain contains all the polymorphisms specifying the peptide binding specificities. Hundreds of DRB1 alleles have been described and typing for these polymorphisms is routinely done for bone marrow and kidney transplantation. HLA-DR is expressed primarily on antigen presenting cells such as B lymphocytes, monocytes, macrophages, thymic epithelial cells and activated T lymphocytes. Three loci, DR, DQ and DP, encode the major expressed products of the human class II region. The human MHC class II molecules bind intracellularly processed peptides, present them to T-helper cells, and have a critical role in the initiation of the immune response.
HLA and MHC antibodies play a significant role in Immunopeptidomics, facilitating the identification and characterization of neoantigens through high-performance liquid chromatography coupled to tandem Mass Spectrometry.

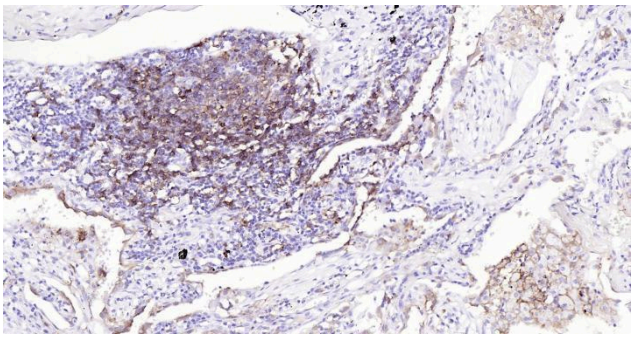
Synonyms

CD74; DHLAG; DR-4; DR4; DRB1; DRB4; DRw10; HLA-DR1B; HLA-DR3B; HLA-DR4B; HLA-DRA; HLA-DRA1; HLA-DRB; HLA-DRB1; HLA-DRB3; HLA-DRB4; HLA-DRB5; HLADG; Ia-GAMMA; II; MLRW; SS1; CD; CELIAC 1; CELIAC1; DP beta 1 chain; DP(W4) beta chain; DPB1; DPB1_HUMAN; DQ A1; FLJ27088; FLJ27328; GSE; HLA class II histocompatibility antigen; HLA class II histocompatibility antigen DR 1 beta; HLA class II histocompatibility antigen DR alpha; HLA class II histocompatibility antigen DR alpha chain; HLA DP histocompatibility type beta 1 subunit; HLA DP1B; HLA DPB1; HLA DQA; HLA DQA1; HLA DQB; HLA DQB1; HLA DR1B; HLA DR3B; HLA DR4B; HLA DRA; HLA DRA1; HLA DRB1; HLA DRB3; HLA DRB4; HLA DRB5; HLA-DPB1; IDDM 1; IDDM1; Major histocompatibility complex class II DP beta 1; Major histocompatibility complex class II DQ alpha 1; Major histocompatibility complex class II DQ beta 1; Major histocompatibility complex class II DR alpha; Major histocompatibility complex class II DR beta 1; Major histocompatibility complex class II DR beta 3; Major histocompatibility complex class II DR beta 4; Major histocompatibility complex class II DR beta 5; MGC117330; MHC class II antigen DPB1; MHC class II antigen DRA; MHC class II HLA DQ alpha 1; MHC class II HLA DR beta 1; MHC class II HLA DR beta 3; MHC DPB1; MHC DQ beta; MHC HLA DPB1; MHC HLA DQ alpha.

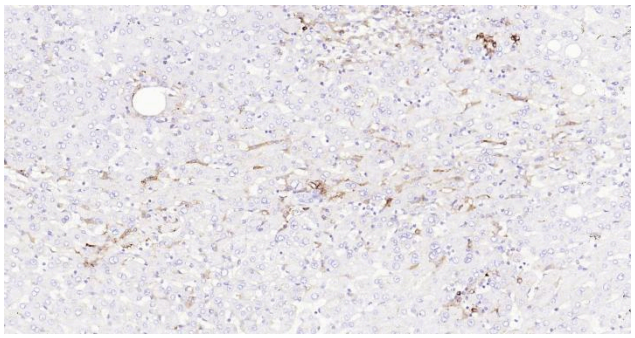
Validation Data



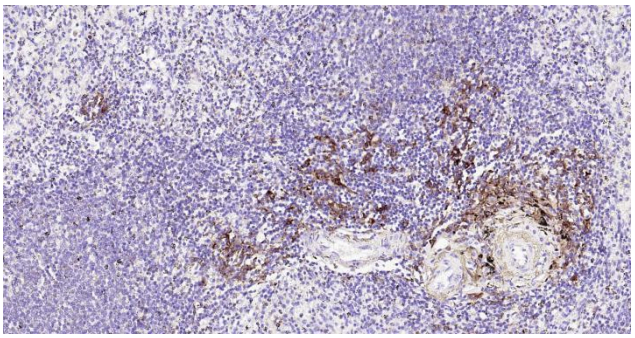
Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0193H (Human HLA-DR IHC Kit).



Immunohistochemical analysis of paraffin embedded human lung cancer tissue slide using IHC0193H (Human HLA-DR IHC Kit).



Immunohistochemical analysis of paraffin embedded human liver tissue slide using IHC0193H (Human HLA-DR IHC Kit).



Immunohistochemical analysis of paraffin embedded human spleen tissue slide using IHC0193H (Human HLA-DR 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 **Human HLA-DR 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** 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 and Slides Mounting

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℃ 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 "IHC0193H, Bioss Antibodies". Citation example: "Human tissue sections using Human HLA-DR IHC Kit (IHC0193H, Bioss Antibodies) were stained for HLA-DR according to the manufacturer's instructions.