Anti-CD20 Antibody [PD00-02]

HA721138

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	IHC-P, mIHC
Molecular Wt:	Predicted band size: 33 kDa
Clone number:	PD00-02

Description: The CD20 antigen is a membrane-embedded, non-glycosylated phosphoprotein, 33-37 kDa. CD20 functions as a Ca2+-permeable cation channel, involved in the regulation of B-cell activation, proliferation and differentiation. CD20 appears on the surface of the pre-B lymphocyte between the time of light chain rearrangement and expression of intact surface immunoglobulin and is lost just before terminal B-cell differentiation into plasma cells. CD20 is virtually specific for normal B-cells. A weak expression has been demonstrated in a subpopulation of T-cells, but not in any other cell type. CD20 is expressed in the large majority of cases of B-cell leukaemia/lymphoma. Early stage precursor B lymphoblastic leukaemia/lymphoma may be negative, and chronic lymphocytic leukaemia/small cell lymphoma may show a weak staining. Plasma cell neoplasms are as a rule CD20 negative. T-cell lymphomas are almost always negative, but CD20 has been demonstrated in few cases of various types of T-cell lymphoma. In Hodgkin lymphoma, the nodular lymphocyte-predominant subtype shows CD20 staining of L&H cells in most cases, while Reed-Sternberg cells in the other subtypes reveal CD20 positivity in about 40, albeit in a minority of neoplastic cells. Acute myeloid leukaemia is CD20 positive in few cases, while blastic transformation in chronic myeloid leukaemia is accompanied by CD20 positivity in about 30%. Thymoma may reveal CD20 positivity in a spindle cell component. In patients treated with retuximab (a humanized anti-CD20 antibody) for malignant B-cell lymphoma, the CD20 epitopes disappear (both in normal and neoplastic B-cells) as a result of down-modulation of CD20 m-RNA in the cells. This process is potentially reversible. Together with CD79a, CD20 is one of the most important markers for the identification of B-cell neoplasms as outlined above. Tonsil and appendix are appropriate controls: The mantle zone B-cells and the germinal centre B-cells must show a very strong staining reaction. No other cells should stain.

Immunogen:	Synthetic peptide within Human CD20 aa 210-297 (Cytoplasmic).
Positive control:	Human tonsil tissue, human lymph nodes tissue, human spleen tissue, human small cell lung cancer, human non-small cell lung cancer, human prostate cancer, human non-small cell lung cancer, mouse spleen tissue.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P11836 Human | P19437 Mouse
Recommended Dilutions:	
IHC-P	1:1,000-1:2,000
mIHC	1:1,500-1:3,000
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

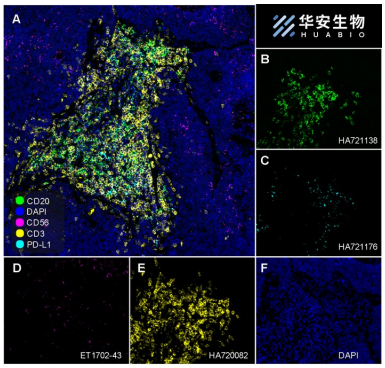

Fig1: Fluorescence multiplex immunohistochemical analysis of Tertiary Lymphoid Structures in Human Small Cell Lung Cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-PD-L1 (HA721176, cyan), anti-CD56 (ET1702-43, magenta) and anti-CD3 (HA720082, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel D: anti-CD56 stained on NKT cells. Panel E: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721176 (1/1,000 dilution), ET1702-43 (1/1,000 dilution), and HA720082 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

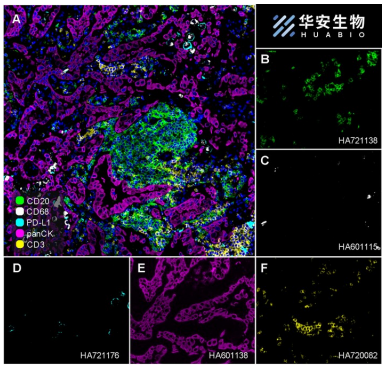

Fig2: Fluorescence multiplex immunohistochemical analysis of the human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD68 (HA601115, gray), anti-PD-L1 (HA721176, cyan), anti-panCK (HA601138, magenta) and anti-CD3 (HA720082, yellow) on human non-small cell lung cancer. Panel B: anti- CD20 stained on B cells. Panel C: anti-CD68 stained on macrophage M1 and macrophage M2. Panel D: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel E: anti-panCK stained on cancer cells. Panel F: anti-CD3 stained on T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA721138 (1/1,500 dilution), HA601115 (1/2,000 dilution), HA721176 (1/1,000 dilution), HA601138 (1/3,000 dilution), and HA720082 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

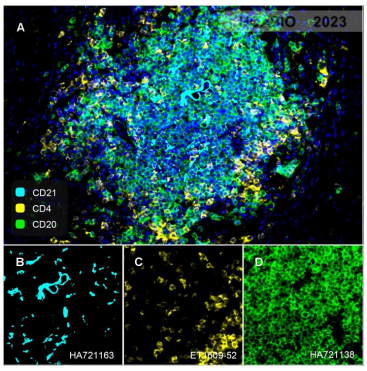

Fig3: Fluorescence multiplex immunohistochemical analysis of tertiary lymphoid structures in human prostate cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD20 (HA721138, green), anti-CD21 (HA721163, cyan) and anti-CD4 (ET1609-52, yellow) on tertiary lymphoid structures. Panel B: anti- CD20 stained on B cells. Panel C: anti-CD21 stained on naive B-cell, memory B-cell and plasma cells. Panel D: anti-CD4 stained on helper T cells and Treg cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of HA721138 (1/1,500 dilution), HA721163 (1/1,000 dilution), and ET1609-52 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

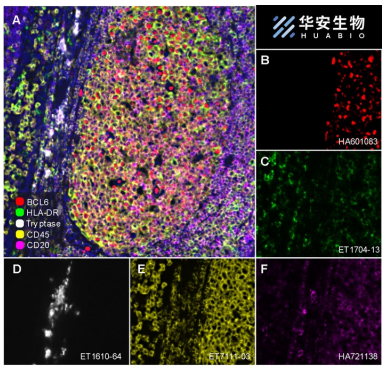

Fig4: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-BCL6 (HA601083, Red), anti-HLA-DR (ET1704-13, Green), anti-Tryptase (ET1610-64, White), anti-CD20 (HA721138, Magenta) and anti-CD45 (ET7111-03, Yellow) on tonsil. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of HA601083 (1/200 dilution), ET1704-13 (1/2,000 dilution), ET1610-64 (1/5,000 dilution), HA721138 (1/2,000 dilution) and ET7111-03 (1/500 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

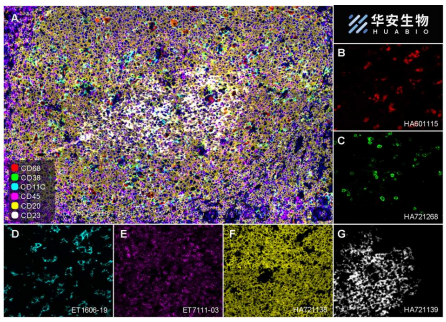

Fig5: Fluorescence multiplex immunohistochemical analysis of Human tonsil (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-CD68 (HA601115, Red), anti-CD38 (HA721268, Green), anti-CD23 (HA721139, White), anti-CD11C (ET1606-19, Cyan), anti-CD45 (ET7111-03, Magenta) and anti-CD20 (HA721138, Yellow) on tonsil. Panel B: anti-CD68 stained on Macrophage. Panel C: anti-CD38 stained on lymphocyte subsets. Panel D: anti-CD11C stained on dendritic cells. Panel E: CD45 stained on lymphocytes. Panel F: anti-CD20 stained on B cells. Panel G: anti-CD23 stained on follicular dendritic cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of HA601115 (1/2,000 dilution), HA721268 (1/1,000 dilution), ET1606-19 (1/1,000 dilution), ET7111-03 (1/500 dilution), HA721138 (1/2,000 dilution) and HA721139 (1/800 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

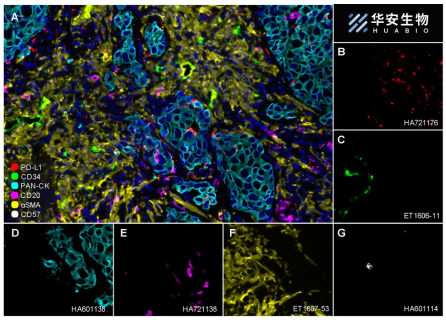

Fig6: Fluorescence multiplex immunohistochemical analysis of Human non-small cell lung cancer (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-PD-L1 (HA721176, red), anti-CD34 (ET1606-11, green), anti-Pan-CK (HA601138, cyan), anti-CD20 (HA721138, magenta), anti-αSMA (ET1607-53, yellow) and anti-CD57 (HA601114, white) on NSCLC. Panel B: anti-PD-L1 stained on dendritic cells and macrophages cells. Panel C: anti- CD34 stained on endothelial cells. Panel D: anti-Pan-CK stained on cancer cells. Panel E: CD20 stained on B cells. Panel F: anti-αSMA stained on cancer-associated fibroblasts and smooth muscle cells. Panel G: anti-CD57 stained on NK cells and T cells. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of HA721176 (1/1,000 dilution), ET1606-11 (1/1,000 dilution), HA601138 (1/3,000 dilution), HA721138 (1/2,000 dilution), ET1607-53 (1/3,000 dilution) and HA601114 (1/1,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

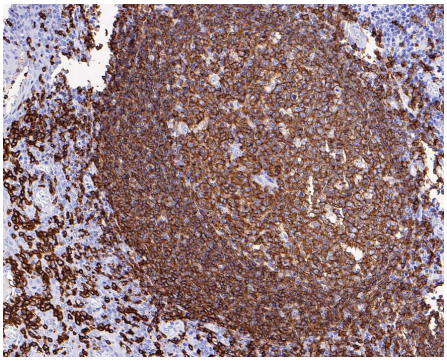

Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD20 antibody (HA721138) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721138) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

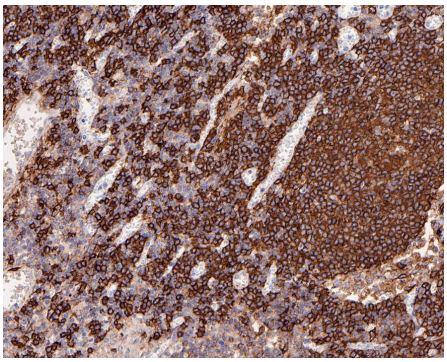

Fig8: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-CD20 antibody (HA721138) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721138) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

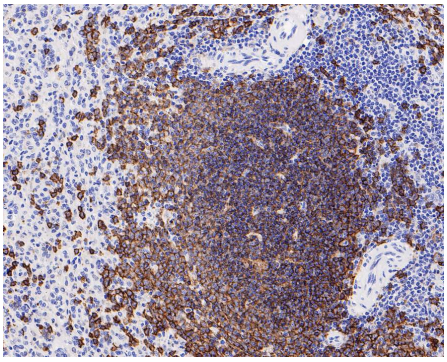

Fig9: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD20 antibody (HA721138) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721138) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

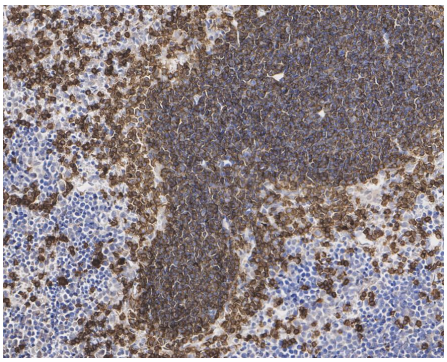

Fig10: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD20 antibody (HA721138) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721138) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Jiang D. et. al. Pyruvate dehydrogenase kinase 4-mediated metabolic reprogramming is involved in rituximab resistance in diffuse large B-cell lymphoma by affecting the expression of MS4A1/CD20. Cancer Sci. 2021 Sep
2. Pavlasova G. et. al. The regulation and function of CD20: an "enigma" of B-cell biology and targeted therapy. Haematologica. 2020 Jun