

## Anti-HLA-C Antibody Picoband™

Catalog Number: PB9377

### About HLA-C

HLA-C belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. Class I molecules play a central role in the immune system by presenting peptides derived from endoplasmic reticulum lumen. They are expressed in nearly all cells. The heavy chain is approximately 45 kDa and its gene contains 8 exons. Exon one encodes the leader peptide, exons 2 and 3 encode the alpha1 and alpha2 domain, which both bind the peptide, exon 4 encodes the alpha3 domain, exon 5 encodes the transmembrane region, and exons 6 and 7 encode the cytoplasmic tail. Polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class one molecule. Typing for these polymorphisms is routinely done for bone marrow and kidney transplantation. Over one hundred HLA-C alleles have been described.

### Overview

Product Name	Anti-HLA-C Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-HLA-C Antibody Picoband™ catalog # PB9377. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q5QT33

### Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human HLA-C.
Predicted Reactive Species	Bovine
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunocytochemistry , 0.5-1ug/ml, Human, -</p> <p>Immunofluorescence, 2ug/ml, Human, -</p> <p>Flow Cytometry, 1-3ug/1x10<sup>6</sup> cells, Human</p>

## Anti-HLA-C Antibody Picoband™ (PB9377) Images

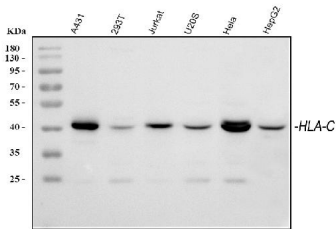


Figure 1. Western blot analysis of HLA-C using anti-HLA-C antibody (PB9377).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human A431 whole cell lysates,  
Lane 2: human 293T whole cell lysates,  
Lane 3: human Jurkat whole cell lysates,  
Lane 4: human U2OS whole cell lysates,  
Lane 5: human HeLa whole cell lysates,  
Lane 6: human HepG2 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-HLA-C antigen affinity purified polyclonal antibody (Catalog # PB9377) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for HLA-C at approximately 41 kDa. The expected band size for HLA-C is at 41 kDa.

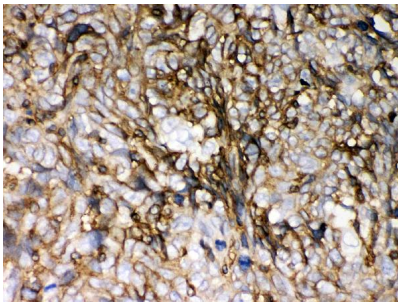


Figure 2 IHC analysis of HLA-C using anti-HLA-C antibody (PB9377).

HLA-C was detected in paraffin-embedded section of Human Lung Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-HLA-C Antibody (PB9377) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

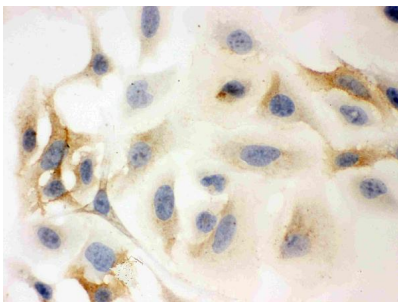


Figure 3. IHC analysis of HLA-C using anti-HLA-C antibody (PB9377).

HLA-C was detected in immunocytochemical section of HELA Cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/ml rabbit anti-HLA-C Antibody (PB9377) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

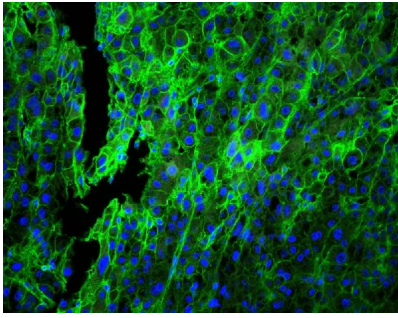


Figure 4. IF analysis of HLA-C using anti-HLA-C antibody (PB9377)

HLA-C was detected in paraffin-embedded section of human Lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution ) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/mL rabbit anti-HLA-C Antibody (PB9377) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

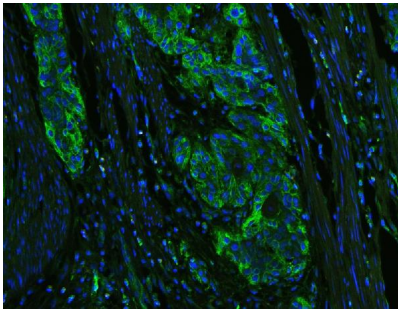


Figure 5. IF analysis of HLA-C using anti-HLA-C antibody (PB9377)

HLA-C was detected in paraffin-embedded section of human oesophagus squama cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution ) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/mL rabbit anti-HLA-C Antibody (PB9377) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

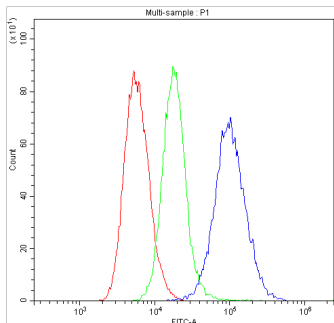


Figure 6. Flow Cytometry analysis of SiHa cells using anti-HLA-C antibody (PB9377).

Overlay histogram showing SiHa cells stained with PB9377 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-HLA-C Antibody (PB9377, 1ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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