

## Anti-Hemoglobin/HBA1/HBA2 Antibody Picoband™

Catalog Number: A00233-1

### About HBA1

The human alpha globin gene cluster located on chromosome 16 spans about 30 kb and includes seven loci: 5'- zeta - pseudozeta - mu - pseudoalpha-1 - alpha-2 - alpha-1 - theta - 3'. The alpha-2 (HBA2) and alpha-1 (HBA1) coding sequences are identical. These genes differ slightly over the 5' untranslated regions and the introns, but they differ significantly over the 3' untranslated regions. Two alpha chains plus two beta chains constitute HbA, which in normal adult life comprises about 97% of the total hemoglobin; alpha chains combine with delta chains to constitute HbA-2, which with HbF (fetal hemoglobin) makes up the remaining 3% of adult hemoglobin. Alpha thalassemias result from deletions of each of the alpha genes as well as deletions of both HBA2 and HBA1; some nondeletion alpha thalassemias have also been reported.

### Overview

Product Name	Anti-Hemoglobin/HBA1/HBA2 Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-Hemoglobin/HBA1/HBA2 Antibody Picoband™ catalog # A00233-1. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na <sub>2</sub> HPO <sub>4</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	P69905

### Technical Details

Immunogen	E.coli-derived human Hemoglobin recombinant protein (Position: V2-R142). Human Hemoglobin shares 85.8% amino acid (aa) sequence identity with mouse Hemoglobin.
Predicted Reactive Species	Chicken
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).
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), 2-5ug/ml, Human, By Heat</p> <p>Flow Cytometry(Fixed), 1-3 ug/1x10<sup>6</sup> cells, Human</p>

## Anti-Hemoglobin/HBA1/HBA2 Antibody Picoband™ (A00233-1) Images

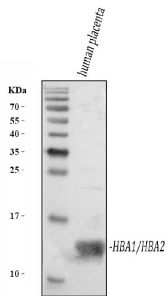


Figure 1. Western blot analysis of Hemoglobin using anti-Hemoglobin antibody (A00233-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human placenta tissue 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-Hemoglobin antigen affinity purified polyclonal antibody (Catalog # A00233-1) 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 Hemoglobin at approximately 15 kDa. The expected band size for Hemoglobin is at 15 kDa.

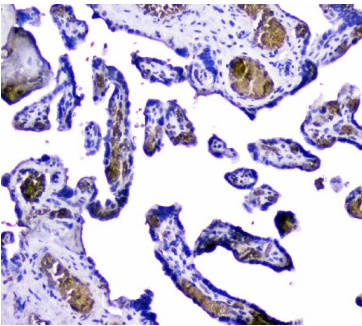


Figure 2. IHC analysis of Hemoglobin using anti-Hemoglobin antibody (A00233-1). Hemoglobin was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Hemoglobin Antibody (A00233-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

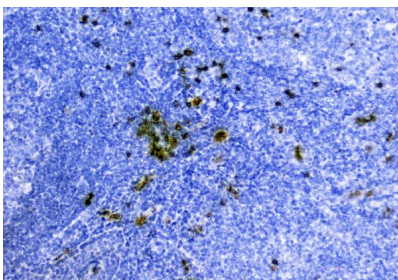
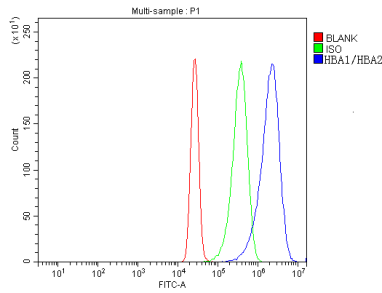


Figure 3. IHC analysis of Hemoglobin using anti-Hemoglobin antibody (A00233-1). Hemoglobin was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Hemoglobin Antibody (A00233-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Figure 4. Flow Cytometry analysis of K562 cells using anti-Hemoglobin antibody (A00233-1).



Overlay histogram showing K562 cells stained with A00233-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Hemoglobin Antibody (A00233-1, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

## 1 Publications Citing This Product

1. PubMed ID: -, Wenxia Su, Lin Yang, Xueping Luo, Meng Chen, Jinqin Liu; Elimination of Autofluorescence in Archival Formaldehyde-Fixed, Paraffin-Embedded Bone Marrow Biopsies. Arch Pathol Lab Med 1 March 2019; 143(3):362-369. doi:https://doi.org/10.5858/arpa.2017-0480-OA

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