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Datasheet for 200-301-GS5S

Hemoglobin beta S Antibody

Overview

Description:	Anti-HbS (MOUSE) Monoclonal Antibody - 200-301-GS5S
Item No.:	200-301-GS5S
Size:	25 µL
Applications:	ELISA, WB, FC, IF, LFA
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	HbS antibodies detect the E6V mutant in the hemoglobin beta subunit. Functional adult hemoglobin (Hb) is a hetero tetramer composed of 2 alpha and 2 beta subunits (α2β2). Common isoform variants of hemoglobin include HbA, HbS, HbC, HbF, and HbA2. Hemoglobin S is the predominant hemoglobin in people with sickle cell disease. The alpha chain is normal. The disease-producing mutation exists in the beta chain, giving the molecule the structure, α2βS2. People who have one sickle mutant gene and one normal beta gene have sickle cell trait which is benign. Globin gene mutations affect the structure and expression levels of Hb. Sickle cell disease and the more benign sickle cell trait are observed in more than 100 million people globally. Perhaps the most significant mutation is the E6V in the beta subunit and the cause of SCD, but other relevant isoforms of Hb are observed. HbS antibody does not react to other forms of Hb. This antibody is ideal for investigators involved in Cardiovascular and developmental biology research.
Synonyms:	mouse anti-HbS antibody, mouse anti-hemoglobin antibody, Hemoglobin beta subunit sickle mutant, HBS, HBBs, HbS Antibody, Sickle Cell Disease (SCD)
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	23E5.H6.G6.C1.H7.F7.G9.F6
Format:	IgG3

Target Details

Gene Name:	HbBs

Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-Hemoglobin beta S Monoclonal Antibody was produced in mice by repeated immunizations with synthetic peptide corresponding to amino acid residues near the N-terminus of Hb β-subunit conjugated to KLH.
Purity/Specificity:	This protein A purified mouse monoclonal antibody reacts specifically with human HbS beta sickle isoform. Anti-HbS is purified from tissue culture supernatant by protein A purification. Blast analysis shows 100% homology to Human, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, and Hylobates lar. This antibody does not react with the HbA, HbF, HbC, or HbA-2 isoform.
Relevant Links:	• 200-301-GS5 SDS • UniProtKB - P68871

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	FC, IF, LFA (Based on references)
Application Note:	Anti-Hemoglobin beta S (MOUSE) antibody has been tested by ELISA and western blot. This antibody is designed for use in lateral flow. Specific conditions of reactivity should be optimized by the end user. Expect a band of approximately 16 kDa in appropriate lysates.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000
WB:	1ug/mL

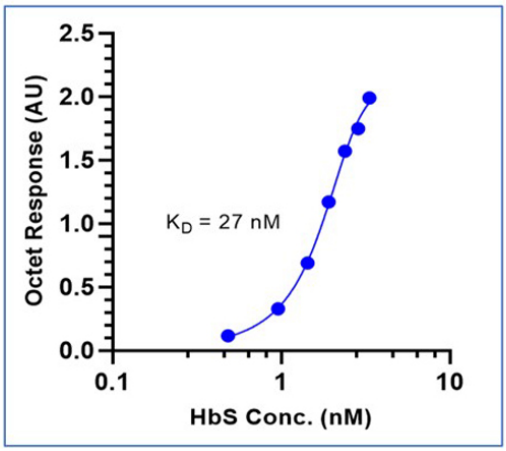
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

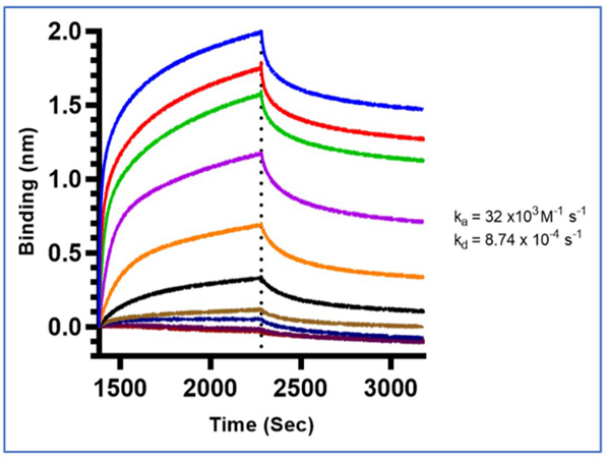
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

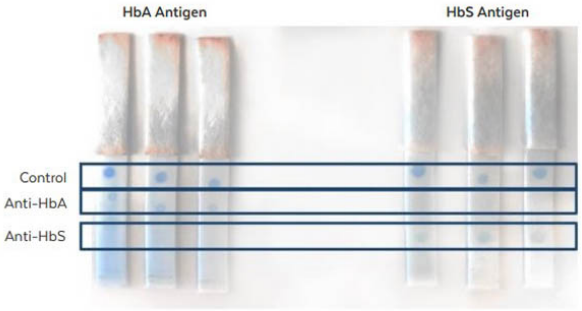
Images



ELISA
Fitting of Kinetic data to bivalent binding site model. Steady state signals (blue filled symbols) are fitted to a bivalent binding site model (blue line). A 4-parameter non-linear regression curve fit was used for the integration of the data. The Mouse Anti-HbS Antibody (p/n 200-301-GS5) presents a KD=27nM (affinity constant) to HbS protein.

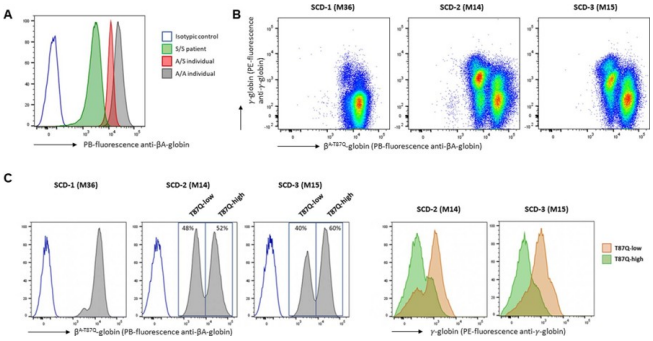


ELISA
Binding mode and kinetic analysis of Mouse Anti-HbS Antibody (p/n 200-301-GS5). Using Octet R4, a series of nine (9) concentrations of Mouse Anti-HbS Antibody (3.0nM-2.2µM) were immobilized on an AR2G biosensor and exposed to 20µg/ml HbS protein. Each colored curve represents the acquired signal of its antibody concentration. The interaction features reproducible duplicate injections and fits to a 1:2 bivalent kinetic model. The association constant (ka=32x103M-1s-1) and dissociation constant (kd=8.74x10-4s-1) values are depicted in the graph. The assay was performed as per the manufacture's protocol.



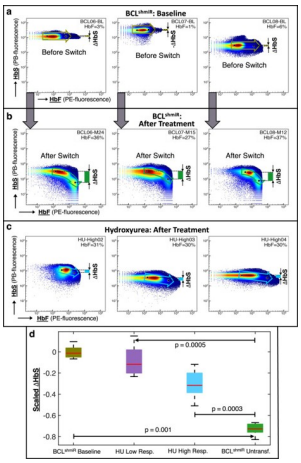
Lateral Flow

Lateral Flow Results of Anti-HbA (Hemoglobin A) and Anti-HbS (Hemoglobin beta S) Antibodies. Triplicate test strips are spotted with a control, Anti-HbA antibody (p/n 200-301-GS4) 0.5µL at 250ug/mL, and Anti-HbS antibody (p/n 200-301-GN5) 0.5µL at 1mg/mL. Recombinant HbA (left group) or recombinant HbS (right group) are observed to react with the corresponding antibodies specifically, leading to blue dots. Image courtesy of team SickIED advised by Professors Xuanhong Cheng and Khanjan Mehta of Lehigh University, Bethlehem, Pennsylvania, USA.



Flow Cytometry

Flow cytometry analyses of RBCs in SCD patients.
(A) Controls showing that the Pacific Blue (PB) labeled anti-HbA monoclonal antibody (Rockland) does not cross react with HbS in individualized RBCs by flow cytometry analysis. S/S RBC exhibit low level of fluorescence because the anti-HbA antibody also reacts with HbA2 (data from Rockland). However, the anti-HbA antibody does not cross react with HbF (data from Rockland). Because the epitope recognized by the anti-HbA antibody is also present on HbAT87Q, the latter is equally well recognized (data not shown). (b) Distribution of γ-globin and βA-T87Q-globin expressing RBCs in SCD patients' blood by flow cytometry using antibodies that recognize either βA-T87Q-globin (anti-HbA PB-labeled) or γ-globin (anti-HbF PE-labeled). Co-stainings performed at M36, M14 and M15 for SCD-1, SCD-2 and SCD-3, respectively, showing reduced levels of HbF in high containing HbAT87Q RBCs, and inversely, for SCD-2 and SCD-3. (c) Distribution of βA-T87Q-globin vs. γ-globin expressing RBCs in SCD patients' blood by flow cytometry using antibodies that recognize either βA-T87Q-globin (left) or γ-globin (right). M, months after GT; PB, Pacific Blue; PE, phycoerythrin. Corresponding datasets are represented in Tables 2a and b. Antibodies used anti-HbA monoclonal antibody (p/n 200-301-GS4) and anti-HbS monoclonal antibody (p/n 301-GS5).
Extended Data Fig. 4.
PMID: 35075288



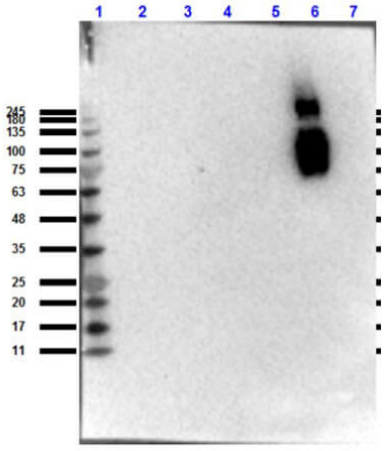
Flow Cytometry

Hb switching after BCL11A inhibition leads to high HbF/low HbS-containing cells.

We investigated the mechanism of Hb switching in BCLshmiR and HU-treated patients by performing intracellular co-staining of single RBCs to assess their relative levels of HbS and HbF. In panels (a–c) the flow cytometry representative dot plots show the HbS fluorescence intensity (y-axis) as a function of HbF fluorescence intensity (x-axis) after intracellular staining for BCLshmiR at baseline (a) and ≥12 months after drug product infusion (b). Panel (c) shows representative measurements performed on HU-treated patients. Flow cytometry data was collected for BCLshmiR- patients at baseline (n = 5), Untransfused BCLshmiR ≥ 12 months after drug product infusion (n = 7), HU High Responders (n = 8), and HU Low Responders (n = 6). Two BCLshmiR baseline blood samples and one HU High Responder blood sample were not available for this assay. For each data sample available, the cells were ordered by HbS fluorescence intensity and clustered into quartiles. The average HbS and HbF fluorescence intensities of each quartile were computed and scaled by dividing the average intensities by their maximum quartile values. Left and right black dots in panels a to c respectively indicate the average of scaled HbF and HbS fluorescence intensities from the 1st and 4th quartiles, while the arrow indicates the Hb switch from the 1st to 4th quartile, and green bars indicate variation in HbS intensities between 4th and 1st quartile. Panel (d) shows boxplots comparing scaled HbS fluorescence intensity between its 4th and 1st quartile for all four cohorts. More than 100,000 cells were collected for each blood sample. Boxplot properties and the method used to compute the p-values in this study are described in the “Methods” subsection “Data analysis, statistics, and reproducibility”. Source data are provided as a Source Data file.

Fig 4.

PMID: 37730674



Western Blot
Western blot results of Mouse Anti-HbS Antibody. Lane 1: Opal Prestained molecular weight ladder - MB-210-0500. Lane 2: HbA. Lane 3: HbA2. Lane 4: HbC. Lane 5: HbF. Lane 6: HbS. Lane 7: BSA. Loaded 10ug. Blocking: BlockOut Universal buffer - MB-073 for 30 min at RT. Primary Antibody: Anti-Hemoglobin beta S at 1:1000 overnight at 4°C. Secondary Antibody: Rabbit Anti-Mouse HRP - 610-403-C46 at 1:40,000 for 30 min at RT.

References

- De Souza DC et al. Genetic reversal of the globin switch concurrently modulates both fetal and sickle hemoglobin and reduces red cell sickling. *Nat Commun.* (2023)
- Chen A et al. Reducing Child Mortality in Sierra Leone with a Sustainable Diagnostics Device for Sickle Cell Disease. *1st International Academic Conference on "WHY IT MATTERS".* (2022)
- Magrin E et al. Long-term outcomes of lentiviral gene therapy for the β-hemoglobinopathies: the HGB-205 trial. *Nature Medicine* (2022)
- Lancia M et al. A Novel E-Junction Lateral Flow Immunoassay for Widespread Sickle Cell Screening in Low and Middle-Income Countries. *IEEE Global Humanitarian Technology Conference (GHTC).* (2020)

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