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Datasheet for 209-4139

Human Red Blood Cell Antibody

Overview

Description:	Anti-Human Red Blood Cell (RBC) (RABBIT) Antibody - 209-4139
Item No.:	209-4139
Size:	50 mg
Applications:	Agglutination, IHC, Biochemical Assay, FC, LFA, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti-Human Red Blood Cells Antibody recognizes human red blood cells and can be used in a variety of agglutination assays where agglutination or clumping of red blood cells is a positive indication of the presence of an analyte, virus particle or bacteria. Red blood cells (RBCs), also known as erythrocytes, are metabolically active cells that are highly adapted to serve their function in blood gas exchange (oxygen/CO2 transport). The red blood cells enable the transport of sufficient O2 between respiratory surfaces (lungs, gills) and metabolizing tissues by means of their high intracellular concentration of hemoglobin.
Synonyms:	rabbit Anti-human RBC antibody, Red blood cell antibody, Antibody for hemagglutination, rabbit anti-human red blood cell, rabbit antibody to human red blood cells (RBC), haemolysin, hemolysin, erythrocytes sensitizing agent, anti-erythrocytes, anti-erythrocytes antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Reactivity:	Human
Immunogen:	Human washed pooled Red Blood Cells (RBC)
Purity/Specificity:	This product is an IgG fraction antibody purified from polyspecific antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above.

Relevant Links:	• 209-4139 SDS

Application Details

Tested Applications:	Agglutination, IHC
Suggested Applications:	Biochemical Assay, FC, LFA, WB (Based on references)
Application Note:	Anti-Human Red Blood Cells Antibody has been tested by agglutination and immunohistochemistry. This product is suitable for sensitizing cells in hemolytic complement assays, and for lymphocyte screening procedures, especially screening for T cells and T receptor-bearing lymphocytes. This product is suitable for conjugation with enzymes, radiolabels, fluorochromes or other markers. Specific conditions should be optimized by researcher.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IHC:	2.5µg/mL
Other:	AGGLUTINATION TITER 1:32 - 1:64

Tissue Data

Tissue Type:	Red Blood Cells

Formulation

Physical State:	Lyophilized
Concentration:	10 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Sterility:	Non-sterile
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None
Reconstitution Volume:	5.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient

Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

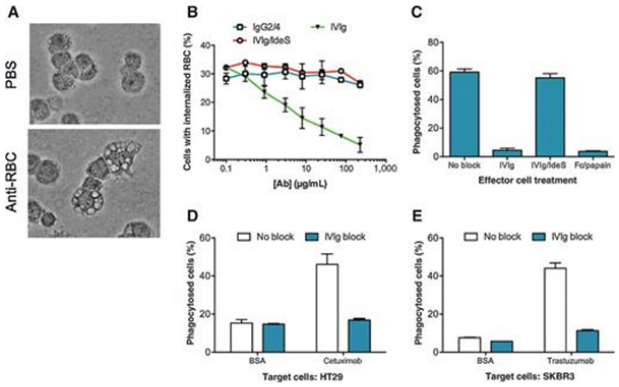
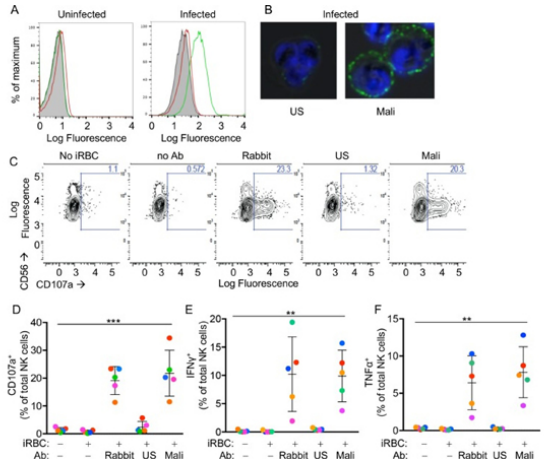
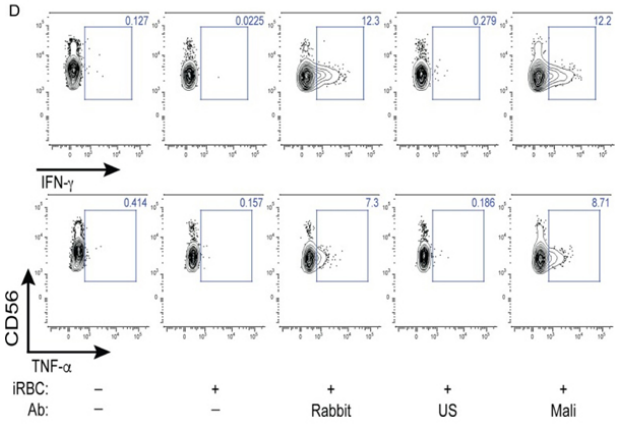


Figure
IdeS treatment enables efficient target specific antibody-mediated phagocytosis by freeing antibody receptors from blocking nontarget-specific antibodies. A, Visualization of engulfed red blood cells (RBC) after opsonization with anti-RBC IgG. B, Effector cells were preincubated with different amounts of IgG being either capable (IVIg), IdeS-treated IVIg (IVIg/IdeS) or engineered to be incapable (IgG2/4) to bind FcγRs. Far Red-labeled target cells (RBC) were opsonized with anti-RBC IgG and mixed with effector cells (THP-1) at 20:1 E:T ratio. C, Calcein-labeled target cells (NuDUL-1) were opsonized with 5 µg/mL alemtuzumab and mixed with Far Red–labeled effector cells (THP-1) at 20:1 E:T ratio in the presence or absence of 100 µg/mL IVIg, IdeS-treated IVIg (IVIg/IdeS) or papain-generated Fc-fragments. D, Calcein-labeled target cells (HT29) were opsonized with 100 µg/mL cetuximab or BSA and mixed with Far Red-labeled effector cells (THP-1) at 20:1 E:T ratio in the presence or absence of 400 µg/mL IVIg. E, Calcein-labeled target cells (SKBR3) were opsonized with 100 µg/mL trastuzumab or BSA and mixed with Far Red–labeled effector cells (THP-1) at 20:1 E:T ratio in the presence or absence of 400 µg/mL IVIg. The percent of phagocytosed target cells was evaluated using flow cytometry. Fig 3. PMID: 28533435



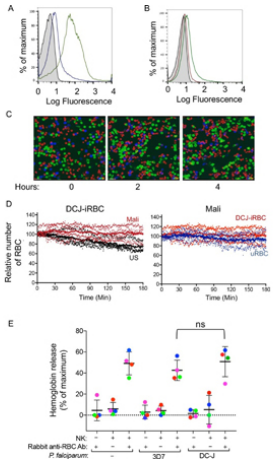
Flow Cytometry

Primary human NK cells are activated by antibody-coated P.f.-iRBCs.
(A) Uninfected RBCs (left) and trophozoite-stage iRBCs (right) were stained with serum pooled from US individuals (red) and plasma pooled from individuals living in a malaria-endemic region of Mali (green). Bound Abs were detected with AF488-conjugated anti-human IgG (H + L) antiserum. (B) Immunofluorescence images of iRBCs stained with DAPI (blue) and with either US serum or Mali plasma, as indicated. Anti-human IgG (H + L) antiserum labeled with AF488 (green) was used to detect Ab-coated RBCs. (C) NK cells stained with PE-Cy5.5-conjugated CD56 and PE-conjugated CD107a Abs. The fraction of CD107a+ NK cells is indicated in each panel. (D) NK cell degranulation measured by CD107a Ab staining. NK cells alone or co-incubated with iRBCs, at a NK:RBC ratio of 1:1 for 4 hr, in the absence or presence of Abs, as indicated. Rabbit polyclonal anti-RBC serum (1:4000), US serum (1:10) and Mali plasma (1:10) were used. Circles indicate individual NK cell donors, each with its own color. Data from independent experiments are shown as mean ± SD (ANOVA, p=0.0009). (E, F) Intracellular staining of IFN-γ and TNF-α. Incubation conditions and Abs as in (D) (ANOVA, p=0.0061 for E, 0.0050 for F). Data from independent experiments are shown as mean ± SD. Figure 1. PMID: 29943728



Flow Cytometry

(D) NK cells alone or co-incubated with iRBCs, at a NK:RBC ratio of 1:1 for 4 h, in the absence or presence of Abs, as indicated. Rabbit polyclonal anti-RBC serum (1:4000), US serum (1:10) and Mali plasma (1:10) were used. After co-incubation NK cells were stained with PE-Cy5.5-conjugated CD56 Ab and with either APC-conjugated IFN-γ or PE-conjugated TNF-α Abs for intracellular cytokines. The fraction of cytokine-positive NK cells is indicated in each panel. Figure 1—figure supplement 1. PMID: 29943728



Flow Cytometry

(A) Immunostaining of uRBCs with Mali plasma (shaded) and of RBCs infected with P.f. 3D7 (green) or with P.f. DC-J parasites (blue). Bound Abs were detected with AF488-conjugated anti-human IgG (H + L) antiserum. (B) Staining of DC-J iRBCs with Abs from US serum (red) and Mali plasma (green). Secondary staining was as in (A). The shaded histogram represents staining with secondary Ab alone. (C) Live imaging of primary NK cells (green) co-incubated with uRBCs (blue) and DC-J-iRBCs (red) at an equal ratio (1:1:1) in the presence of US serum (1:10) or Mali plasma (1:10). Cell counts for NK cells, DC-J-iRBCs and uRBCs were determined every minute for 3 hr. Representative snapshots taken at time 0, 2 and 4 hr are shown. (D) Cell numbers were normalized to 100 at the start of image acquisition. Composite display of 4 independent experiments, each carried out with a different NK cell donor (dotted lines). The mean is shown as a solid line. (E) NK cell-mediated ADCC towards uRBCs, P.f. 3D7 iRBCs and P.f. DC-J iRBCs. Cells were mixed at an NK:RBC ratio of 5:1 and incubated for 5 hr in the presence or absence of rabbit anti-RBC serum (1:4000), as indicated. Hemoglobin release, measured using a Quantichrom Hb assay, is shown relative to release from RBCs treated with 1% Triton X-100. Data are shown (mean ± SD) for NK cells from four independent donors (t-test, p=0.3743, comparing 3D7 and DC-J in presence of NK cells and Rabbit anti RBC Ab). Figure 3. PMID: 29943728

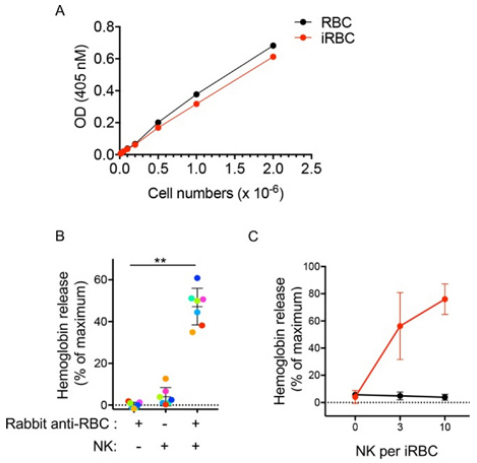


Figure
Hemoglobin release assay.
(A) Hemoglobin in supernatants of RBCs treated with 1% Triton X-100 was measured with a Quantichrom assay kit. Graph shows OD versus cell number from a representative experiment with uRBCs (black) and iRBCs (red). (B) Hemoglobin in the supernatant of iRBCs incubated for 5 hr in the presence or absence of rabbit anti-RBC serum (1:4000) and of NK cells, as indicated. Cells were mixed at an NK per iRBC ratio of five. Data are shown (mean ± SD) for NK cells from seven independent donors, relative to maximum hemoglobin release in 1% Triton X-100 (ANOVA, p<0.0001). (C) Hemoglobin in the supernatant of iRBCs incubated for 5 hr at different numbers of NK per iRBC in the presence (red) or absence (black) of rabbit anti-RBC serum (1:4000). Data are shown (mean ± SD) for NK cells from three independent donors (t test, p (0)=0.576; p (3)=0.022; p (10) = 0.0003).
Figure 3—figure supplement 1.
PMID: 29943728

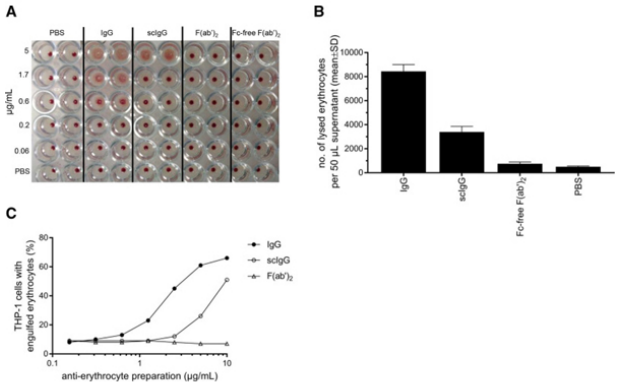
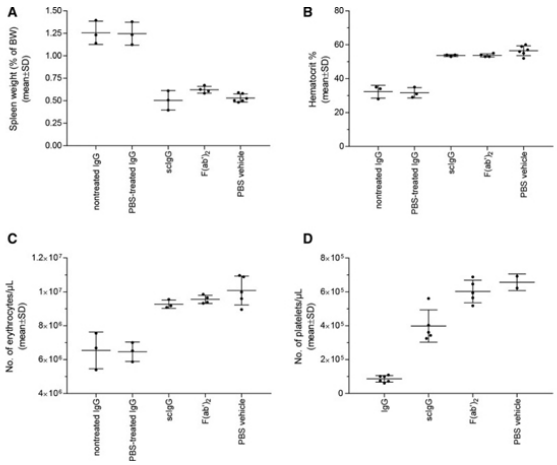
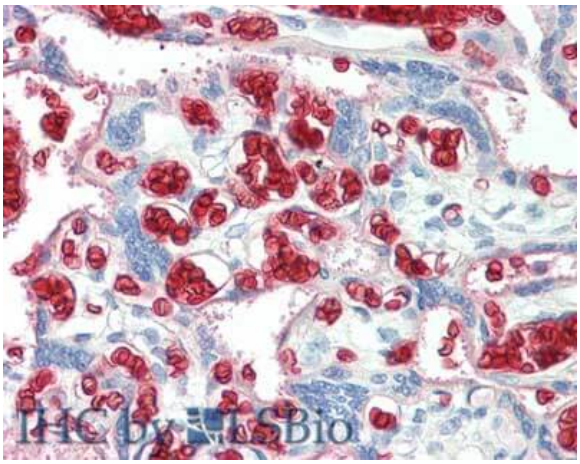


Figure
Complement-independent effector functions of imlifidase-generated anti-erythrocyte fractions. In (A), FcγR-dependent retention of erythrocytes to a confluent layer of THP-1 cells after opsonization with anti-erythrocyte IgG, scIgG, or F (ab')2 in the absence of complement (n = 2). In (B), ADCC activity of THP-1 cells after opsonization of erythrocytes with the different anti-erythrocyte IgG fractions (n = 2–3). In (C), ADCP activity of THP-1 cells after opsonization with the different anti-erythrocyte IgG fractions. For more information on the IgG fractions used, see Figure S4, SDC, http://links.lww.com/TP/C327. ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; IgG, immunoglobulin G; MFI, mean fluorescence intensity; scIgG, single-cleaved IgG. Fig 6.
PMID: 34966107



Western Blot
FcγR-dependent eAIHA (A–C) and eITP (D) in mice induced with the anti-erythrocyte/antiplatelet preparations shown in Figure S5, SDC, http://links.lww.com/TP/C327. In eAIHA, spleen weights (A), hematocrit (B) and erythrocyte number (C) were assessed at termination (day 3). In eITP, platelets were counted 1 d after disease induction (D). eAIHA, experimental autoimmune hemolytic anemia; eITP, experimental immune thrombocytopenic purpura; IgG, immunoglobulin G; scIgG, single-cleaved IgG. Fig 7.
PMID: 34966107



Immunohistochemistry
Immunohistochemistry of Human Red Blood Cell antibody. Tissue: placenta. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: Human Red Blood Cell antibody at 2.5 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rat secondary antibody at 1:10,000 for 45 min at RT. Staining: Human Red Blood Cell as precipitated red signal with hematoxylin purple nuclear counterstain.

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