

**Datasheet for R406-0050****Sheep Red Blood Cells 100% Washed Pooled Cells****Overview**

<b>Description:</b>	Sheep Red Blood Cell (RBC) 100% Washed Pooled Cells - R406-0050
<b>Item No.:</b>	R406-0050
<b>Size:</b>	50 mL
<b>Applications:</b>	Functional Assay, IF, Other
<b>Origin:</b>	Sheep

**Product Details**

<b>Background:</b>	Sheep red blood cells are useful for the titration of complement, adsorption procedures, testing for agglutinins/HA assays, and for the preparation of stroma as particulate reagents.
<b>Synonyms:</b>	Sheep Washed Pooled Cells, Sheep WPCs, Sheep Red Blood Cells, Sheep RBCs, sheep erythrocytes
<b>Species of Origin:</b>	Sheep

**Target Details**

<b>Purity/Specificity:</b>	Sheep whole blood is washed to remove the platelet rich plasma, buffy coat layer, and leukocytes (WBC). After processing, the finished product is supplied as 100% red blood cells. Sheep red blood cells are perishable and are collected and processed upon receipt of your order.
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**Application Details**

<b>Suggested Applications:</b>	Functional Assay, IF, Other (Based on references)
<b>Application Note:</b>	Complement titration, adsorption procedures, HA assays and for the preparation of stroma as particulate reagents.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

## Tissue Data

<b>Tissue Type:</b>	Red Blood Cells
<b>Sex:</b>	Mixed
<b>Strain:</b>	Sheep - Mixed

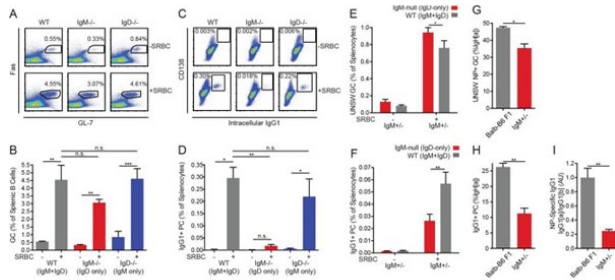
## Formulation

<b>Physical State:</b>	Liquid
<b>Buffer:</b>	None
<b>Sterility:</b>	Non-sterile
<b>Preservative:</b>	None
<b>Stabilizer:</b>	None

## Shipping & Handling

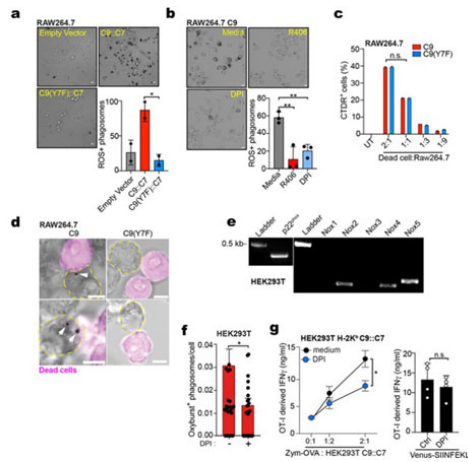
<b>Shipping Condition:</b>	Wet Ice
<b>Storage Condition:</b>	Store sheep washed pooled red blood cells at 4° C prior to opening. Be advised that blood is a perishable product and exact shelf may depend on application.
<b>Expiration:</b>	This product MAY be stable for up to one (1) week if properly stored and handled.

## Images



## Figure

IgD-only cells have intact germinal center responses but impaired IgG1+ SLPC responses. (A) Splenic (CD19<sup>+</sup>) B cells from WT, IgM<sup>-/-</sup>, and IgD<sup>-/-</sup> mice unimmunized or 5 days after i.p. immunization with 200  $\mu$ L of 10% SRBCs. (B) Quantification of germinal center (Fas<sup>hi</sup> GL-7<sup>hi</sup>) cells in (A). (C) Splenocytes from mice in (A). (D) Quantification of CD138<sup>+</sup> IgG1<sup>+</sup> plasma cells in (C). (E) WT (IgM<sup>b+</sup>) and IgM-null (IgD<sup>a+</sup>) germinal center B cells as a percentage of live splenocytes in unimmunized and IgM<sup>+/+</sup> mice 5 days after i.p. immunization with 200  $\mu$ L of 10% SRBCs. (F) WT (IgG1<sup>b+</sup>) and IgM-null (IgG1<sup>a+</sup>) switched plasma cells (CD138<sup>+</sup> IgG1<sup>+</sup>) as a percentage of live splenocytes in IgM<sup>+/+</sup> mice unimmunized or 5 days after i.p. immunization with 200  $\mu$ L of 10% SRBCs. (G) Fraction of unswitched NP-specific germinal center cells (CD19<sup>+</sup> Fas<sup>hi</sup> GL-7<sup>hi</sup> IgM/IgD<sup>+</sup>) from the IgHa locus in the spleens of Balb/c-B6 F1 and IgM<sup>+/+</sup> mice 7–8 days after i.p. immunization with 100  $\mu$ g NP-RSA. (H) Fraction of IgG1<sup>+</sup> CD138<sup>+</sup> plasma cells from the IgHa locus in Balb/c-B6 F1 and IgM<sup>+/+</sup> mice 7–8 days after i.p. immunization with 100  $\mu$ g NP-RSA. (I) NP-specific IgG1a and IgG1b titers at OD = 0.2 were calculated for the mice in (G–H) by ELISA. The IgG1a to IgG1b titer ratio was calculated for each mouse, and all ratios were normalized such that the average IgG1a/IgG1b ratio in Balb/c-B6 F1 samples = 1.0. For (A–D), statistics from  $n = 4$  unimmunized mice of each genotype and  $n = 3$  WT,  $n = 6$  IgM<sup>-/-</sup>, and  $n = 7$  IgD<sup>-/-</sup> immunized mice were pooled. For (E–F),  $n = 5$  unimmunized and  $n = 5$  immunized mice are shown. For (G–I),  $n = 5$  Balb/c-B6 F1 mice and  $n = 3$  IgM<sup>+/+</sup> mice are shown. One-way ANOVA with Tukey's multiple comparisons test (B and D), a paired t test (E–F), and Welch's t test (G–I) were used to calculate p values, and mean  $\pm$  SEM is displayed. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Figure 7. PMID: 29521626.



## Figure

DNGR signalling promotes phagosomal ROS production. a-b, Confocal images of RAW264.7 cells transfected with empty vector or plasmid encoding C9::C7 or C9(Y7F)::C7 receptors and pulsed with zymosan (a) or dead sRBCs (b) in the presence of Nitroblue tetrazolium (NBT) (Scale bar 10  $\mu$ m). Quantification of ROS+ phagosomes. Data represented as mean ( $\pm$  s.e.m.) (a) or ( $\pm$  s.d.) (b) and are representative of two independent determinations ( $n = 2$ ). P values determined by one-way ANOVA. c, RAW264.7 stably expressing C9 or C9(Y7F) receptors were pulsed with CellTracker DeepRed (CTDR)-labelled FP-sRBCs for 2 hrs. Percentage of CTDR+ RAW264.7 cells was quantified by flow cytometry. Data represented as mean ( $\pm$  s.d.) and are representative of two independent experiments ( $n = 2$ ). d, Confocal images of RAW264.7 stably expressing C9 or C9(Y7F) receptors pulsed with dead cells in the presence of NBT for 2 hrs (scale bars 10  $\mu$ m). Image is a representative image of three similar images. e, RT-PCR of NADPH oxidase subunits in HEK293T. Representative of two experiments ( $n = 2$ ). f, HEK293T cells stably expressing C9::C7 were challenged with zymosan-Oxyburst in the presence or absence of DPI for 1 hr. Oxyburst+ positive phagosomes were quantified across 5 fields of view ( $n > 100$  phagosomes). Data represented as mean ( $\pm$  s.e.m.). P values were calculated by unpaired parametric test, Mann-Whitney and are representative of two independent experiments ( $n = 2$ ). g, HEK293T C9::C7 cells were pulsed with zymosan-Ova (left) or transfected with plasmid encoding VENUS-SIINFELK (right) in the presence or absence of DPI (10  $\mu$ M) for 4 hrs before fixing and adding of OT-I Rag1  $^{-/-}$  T-cells. IFN- $\gamma$  was assessed by ELISA, plotted as mean ( $\pm$  s.d.) of an experimental triplicate. n.s., not significant; \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ . Extended Data Fig 6. PMID: 33349708.

## References

- Schultz JR et al. Identification of 5-(Aryl/Heteroaryl)amino-4-quinolones as Potent Membrane-Disrupting Agents to Combat Antibiotic-Resistant Gram-Positive Bacteria. *J Med Chem.* (2022)
- Canton, J et al. The receptor DNGR-1 signals for phagosomal rupture to promote cross-presentation of dead-cell-associated antigens. *Nature Immunology* (2021)
- Noviski, M et al. IgM and IgD B cell receptors differentially respond to endogenous antigens and control B cell fate. *ELife* (2018)
- Eisenstein TK et al. Anandamide and  $\Delta^9$ -tetrahydrocannabinol directly inhibit cells of the immune system via CB2 receptors. *J Neuroimmunol.* (2007)

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