

**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. |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**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   |

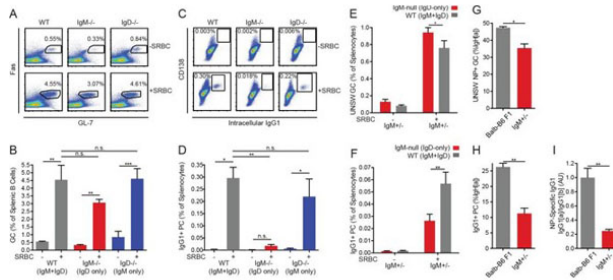
## Formulation

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

## Shipping & Handling

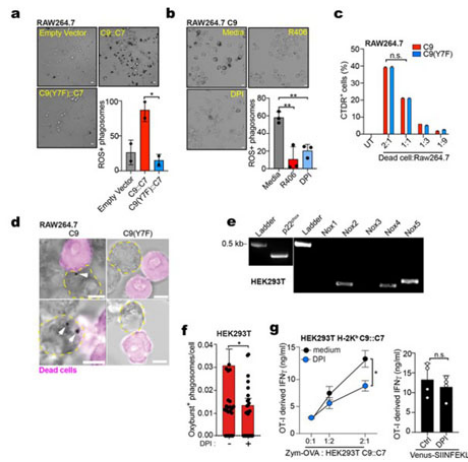
|                            |                                                                                                                                                              |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <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 μL 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 μL 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 μL 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 μg 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 μg 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 + 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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