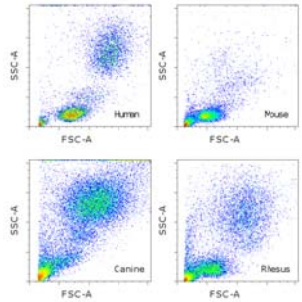
eBioscience™ 10X RBC Lysis Buffer (Multi-species)

Catalog Number: 00-4300

For Research Use Only. Not for use in diagnostic procedures.



Lysis of normal human, mouse, canine, and rhesus peripheral blood. Total viable cells were used for analysis.

Product Information

Contents: eBioscience™ 10X RBC Lysis Buffer (Multi-species)

REF **Catalog Number:** 00-4300

Formulation: aqueous buffer, no sodium azide

Temperature Limitation: Store at 2-25°C.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Description

This 10X RBC Lysis Buffer (Multi-species) is specially formulated for optimal lysis of erythrocytes in single-cell suspensions of peripheral blood and hematopoietic tissues such as spleen. This buffer can be used for lysis of human, mouse, rat, canine, and non-human primate samples. 10X RBC Lysis Buffer (Multi-species) contains ammonium chloride, which lyses red blood cells with a minimal effect on lymphocytes when used as instructed.

Applications Reported

10X RBC Lysis Buffer (Multi-species) has been reported for use in flow cytometric analysis, and cell culture.

Applications Tested

The 10X RBC Lysis Buffer (Multi-species) has been tested on normal human, mouse, rat, canine, and rhesus peripheral blood followed by flow cytometric analysis. This is a 10X solution and should be diluted to 1X using reagent-grade water. Use the 1X solution within 1 month of preparation.

Related Products

00-4222 eBioscience™ Flow Cytometry Staining Buffer
00-4333 eBioscience™ 1X RBC Lysis Buffer
00-5333 eBioscience™ 1-step Fix/Lyse Solution (10X)

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

14 March 2017 Rev. 10

invitrogen

10X RBC Lysis Buffer (Multi-species)

General Protocols: 10X RBC Lysis Buffer (Multi-species)

Materials Needed

- 10X RBC Lysis Buffer (Multi-species) (Cat. No. 00-4300)
- 12x75 mm round bottom test tubes
- Primary antibodies (directly conjugated)
- Flow Cytometry Staining Buffer (Cat. No. 00-4222)

General Experimental Procedure

Before using, Thermo Fisher 10X RBC Lysis Buffer (Multi-species) must be diluted 1:10 with room temperature reagent grade water.

Antibody staining followed by lysis of whole peripheral blood

Note: *Refer to bulk lysis protocol (last protocol) for RBC lysis before antibody staining*

1. Aliquot a sample of whole blood into a tube.
   For human, use 100 µL of blood.
   For mouse, use 50 – 100 µL of blood.
   For rat, use 50 – 100 µL of blood.
   For canine, use 100 µL of blood.
   For non-human primate, use 100 µL of blood.
   Note: *The Thermo Fisher 10X RBC Lysis Buffer (Multi-species) has been shown to work equivalently in blood collected with either heparin or ETDA as the anticoagulant.*
2. Add the antibody(s) needed for staining (in a volume no greater than 50 µL) and mix thoroughly.
3. Incubate for 30 minutes in the dark (if staining with fluorochrome-conjugated antibodies) at room temperature.
4. Add 2 mL of room temperature prepared 1X RBC Lysis Buffer (Multi-species), and then pulse vortex or invert to mix.
5. Incubate at room temperature in the dark.
   For human, incubate for 10 – 15 minutes.
   For mouse, incubate for 4 – 10 minutes.
   For rat, incubate for 4 – 10 minutes.
   For canine, incubate for 10 – 15 minutes.
   For non-human primate, incubate for 10 – 15 minutes.
   Note: *Turbidity can be observed to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.*
6. After lysis, centrifuge immediately at 500 x *g* for 5 minutes at room temperature. Decant the supernatant.
7. (Optional) The samples can again be incubated with additional 1X RBC Lysis Buffer (Multi-species) (1 mL for 3 minutes) if further removal of red blood cells is needed. However, this step is not typically necessary since small numbers of residual red blood cells do not interfere with subsequent assays and can be gated out during flow cytometric analysis.
8. Resuspend the pellet in 2 mL of Flow Cytometry Staining Buffer and centrifuge again.
9. Decant the supernatant and resuspend the cell pellet in 200 µL of Flow Cytometry Staining Buffer.
10. Analyze the samples by flow cytometry.

Lysis of mouse or rat spleen and bone marrow cells:

1. Harvest mouse spleen and prepare a single-cell suspension.
2. Pellet the cells by centrifugation at 500 x *g* for 5 minutes at room temperature and aspirate the supernatant.
3. Resuspend the pellet in 3-10 mL of prepared 1X RBC Lysis Buffer (Multi-species).
4. Incubate for 4 - 5 minutes at room temperature.
5. After lysis, centrifuge immediately at 500 x *g* for 5 minutes at room temperature. Decant the supernatant.
6. Resuspend the pellet in 2 mL of Flow Cytometry Staining Buffer and centrifuge again.
7. Decant the supernatant and perform a cell count at this time.

Bulk lysis of whole blood:

1. Add 10 mL of prepared 1X RBC Lysis Buffer (Multi-species) per 1 mL of human blood.

   Note: *If cells are to be put in culture, perform using asceptic techniques.*

2. Incubate for 10-15 minutes at room temperature (no more than 15 minutes).

   Note: *Turbidity can be observed to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.*

3. Centrifuge at 300-400 x *g* at room temperature. Decant the supernatant and resuspend the pellet in the appropriate buffer for use in the next step of your experimental procedure.

4. Perform a cell count at this time.

   Note: *In general a small number of residual red cells does not interfere with the proliferation and can be gated out from subsequent flow cytometric analysis. However, if required, a second round of lysis can be performed.*

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288