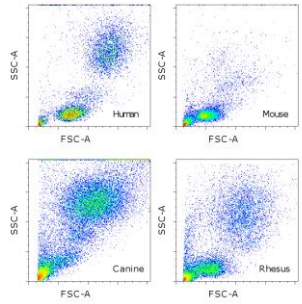
# 10X RBC Lysis Buffer (Multi-species)

**Catalog Number:** 00-4300
**GPR: General Purpose Reagents. For Laboratory Use.**




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

## Product Information

**Contents:** 10X RBC Lysis Buffer (Multi-species)
REF **Catalog Number:** 00-4300
**Handling Conditions:** Use the 1X solution within 1 month of preparation.

**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.

## Related Products

00-4222 Flow Cytometry Staining Buffer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## Protocol: Staining and lysing with 10X RBC Lysis Buffer (Multi-species)

### Materials

- 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)
- eFluor® NC Flow Cytometry Staining Buffer (Cat. No. 00-3222) – for staining with eFluor® NC conjugated antibodies.

### Experimental Procedure

Before using, the 10X RBC Lysis Buffer (Multi-species) must be diluted by adding 1 part 10X RBC Lysis Buffer with 9 parts room temperature reagent grade water.

**Staining and lysis of whole peripheral blood for flow cytometric analysis:**

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 10X RBC Lysis Buffer (Multi-species) has been shown to work equivalently in blood collected with either heparin or ETDA as the anti-coagulant.
2. Add the antibody(s) needed for staining (in a volume no greater than 50 µL) and mix thoroughly.
3. Incubate for 30 min 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 min.
   For mouse, incubate for 4 – 10 min.
   For rat, incubate for 4 – 10 min.
   For canine, incubate for 10 – 15 min.
   For non-human primate, incubate for 10 – 15 min.
   *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 min 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



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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 approximately 200 µL of flow stain buffer.
10. Analyze the samples by flow cytometry.

**Lysis of mouse/rat spleen or bone marrow cells:**

1. Harvest tissue and prepare a single-cell suspension.
2. Pellet the cells by centrifugation at 500 x *g* for 5 min 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 min at room temperature.
5. After lysis, centrifuge immediately at 500 x *g* for 5 min at room temperature. Decant the supernatant.
6. Resuspend the pellet in 2 mL of Flow Cytometry Staining Buffer or buffer of choice 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 min 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.



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