Mouse Erythrocyte Lysing Kit

Catalog Number: WL2000

INTENDED USE

The Mouse Erythrocyte Lysing Kit is designed to gently lyse erythrocytes from murine spleen cell preparations and stabilize the cells with fixative for later analysis by flow cytometry. Cells exposed to the lysing reagent retain their natural light scattering characteristics and fluorescent staining by flow cytometry. This erythrocyte lysing method also has an advantage over other lysing methods in that the viability of unfixed cells is maintained. The fixative and lysing reagents are provided as separate reagents, thus, allowing cells to be used in tissue culture following RBC lysis.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 20-25 °C. **DO NOT FREEZE.**

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
M-Lyse Buffer (10X)	895049	50 mL of a 10-fold concentrated buffer.	May be stored for up to 1 year 20-25 ºC.*
Wash Buffer (10X)	895940	100 mL of 10-fold concentrated buffer.	
Fixative (10X)	895941	25 mL of a formaldehyde solution. *Use undiluted.*	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Deionized or distilled water
- 15 mL or 50 mL cetrifuge tubes
- Hanks' BSS Media
- Bovine serum

REAGENT PREPARATION

1X M-Lyse Buffer - Add 1 mL of M-Lyse Buffer (10X) to 9 mL of distilled water to prepare 10 mL of 1X M-Lyse Buffer.

1X Wash Buffer - Add 1 mL of Wash Buffer (10X) to 9 mL of distilled water to prepare 10 mL of 1X Wash Buffer.

PROCEDURE

1. Gently tease apart the mouse spleen(s) to generate a single cell suspension in Hanks' BSS + 10% serum. Wash the cells once by filling a 15 or 50 mL centrifuge tube with Hanks' BSS + 10% serum and centrifuging the cells for 10 minutes at 200 x g (use a 50 mL tube when processing more than 2 spleens).
2. Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in 1X M-Lyse Buffer and quickly vortex the tube. **Note:** *We recommend using 2 mL of 1X M-Lyse Buffer per processed spleen (approximately 1 mL of 1X M-Lyse Buffer per 25 million splenocytes).*
3. Incubate the cells at room temperature until red blood cell lysis is complete (10 minutes). This is easily observed by a darkening in the color of the fluid and clearing of turbidity. Exposure of the cells to M-Lyse Buffer for longer periods (*i.e.* 30 minutes) will reduce cellular viability and decrease the total yield of leukocytes.
4. Wash the cells by filling the tube with 1X Wash Buffer and centrifuging the cells for 10 minutes at 200 x g. Resuspend the cells in 1X Wash Buffer or culture media.
5. If flow cytometric analysis of the cells will be delayed for more than 1 hour, the cells can be fixed at this time to stabilize them for later analysis. This step should be eliminated if cells are to be used in culture. Add 100 µL of Fixative (10X) to 1 mL of the cells resuspended in wash buffer and then vortex the suspension. Cells should be stored at 2-8 °C until analysis. Although stained cells will be stable for up to 48 hours, we recommend that flow cytometric analysis be performed as soon as possible.