RayBio® Intracellular Staining Kit

User Manual Version 1.0
Oct 31st, 2018

Intracellular Kit Protocol

Catalog numbers: 137-00007-100 (100 tests)

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RayBiotech, Inc.

RayBio® Intracellular Staining Kit

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I. INTRODUCTION

The RayBiotech Intracellular Staining Kit is designed to allow for intracellular staining of target cytokines, proteins, or other factors in activated or treated cell populations. The kit includes both Brefeldin A and Monensin both of which inhibit the Golgi apparatus function, trapping normally secreted cytokine and proteins inside cells during incubation. Following incubation, the included saponin-containing buffer is used to permeabilize cells in order to allow intracellular antibodies entry through the plasma membrane. Your antibodies of interest can then be used to stain these trapped proteins inside the cell directly.

II. KIT CONTENTS

Components	137-00007-100	137-0007-500	Part Number
	100 tests		
Fixation buffer, 4% paraformaldehyde	10 mL	50 mL	Item A
RayBio® Cell Permeabilization Buffer, 10x	20 mL	100 mL	Item B
Brefeldin A, 1000x	100 µL	500 µL	Item C
Monensin, 1000x	100 µL	500 µL	Item D

- Kit can be stored at 4°C, protected from light for up to 3 months. Brefeldin A and Monensin can be stored as aliquots in -20°C for extended time.
- Create enough 1x permeabilization buffer for your number of samples by diluting the 10X concentrate with ddH2O prior to the assay. Store the stock 10x concentrate back at 4°C.

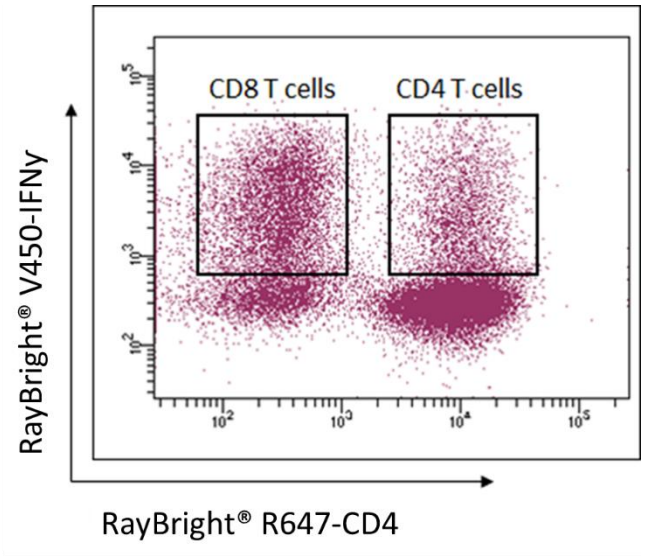
III. Assay Protocol

1. Treat or activate your cells in culture in the presences of the Golgi inhibitors Brefeldin A (Item C) or monensin (Item D).
2. Transfer cells to 96-well plate, and wash cells with 200 µL cold PBS, and spin down at 450g for 10 minutes.
3. (optional) If cells are to be labeled with surface stains, proceed with surface staining of fluorochrome conjugated antibodies per your desired protocol in FACS buffer.
4. After surface staining (if performed), wash cells with 200 µL cold PBS, spin down at 450g for 10 minutes, and remove supernatant.
5. Fix cells by adding 100 µL Fixation Buffer (Item A) for 15 minutes at room temperature.
6. Spin down the cells and resuspend cells in 200 µL of RayBio® Cell Permeabilization Buffer (Item B).
7. Incubate the cells on ice or at 4°C for 15 minutes, then spun down the cells at 450g for 10 minutes.
8. Repeat step 6, 7 once

9. Resuspend cells in 50 µL of RayBio® Cell Permeabilization Buffer containing pre-titrated fluorochrome conjugated antibody(s) against cytokines or other intracellular proteins. Incubate for 30 minutes on ice or at 4°C.
10. Wash cells with 200 µL of RayBio® Cell Permeabilization Buffer twice and resuspend cells in FACS buffer for analysis.
11. Analyze by flow cytometry.

Representative Data:

- One million mouse splenocytes were stimulated by Ionomycin (25 ng/ml) and PMA (1 µg/ml) for 5 hours in vitro in the presence of Brefeldin A (included in the kit). Cells were processed with RayBio® Intracellular staining kit per the recommended protocol. Cells were stained with RayBright® R647 anti-mouse CD4 and RayBright® V450 anti-mouse IFNγ at 1:50 dilution in RayBio® Cell Permeabilization Buffer.



IV. Storage and Stability

Store kit at 4°C, protected from light for up to three months for full functionality

This product is for research use only.