



Phagosome Staining Kit

User Manual

Catalog # CAK2013

(Version 1.1A)

For selectively staining the phagosome in live or aldehyde fixed cells

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Phagosome Staining Kit provides a simple and rapid way to stain phagosome in live cells, or aldehyde-fixed cells selectively. Lucifer Yellow Cadaverine can label endocytic vesicles in cells. Owing to its high aqueous solubility, bright fluorescence, low cytotoxicity, and relatively large Stokes shift.

II. PRINCIPLES

Lucifer Yellow carries two sulfonic acid groups, rendering it membrane-impermeant and incapable of forming covalent bonds with proteins. When Lucifer Yellow is present in the extracellular medium, cells internalize it into fluid-phase vesicles via pinocytosis or macropinocytosis. During particle phagocytosis, if Lucifer Yellow is present extracellularly at the time of engulfment, it becomes trapped within the sealed phagosome lumen. After washing away extracellular Lucifer Yellow with ice-cold PBS, only Lucifer Yellow enclosed within intracellular membranes is retained.

III. KIT COMPONENTS

Component	100 Assays	Storage
Lucifer Yellow	Powder x 1	-20 °C
Inhibitor	Powder x 1	-20 °C
Inhibitor Diluent	0.05 ml x 1	RT
Diluent	10 ml x 1	4 °C
Zymosan	Powder x 2	-20 °C
Zymosan Resuspension	3 ml x 1	-20 °C
Wash Buffer	30 ml x 3	4 °C
Fixation Solution	10 ml x 1	4 °C
Manual	1	

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Standard fluorescence microscope
2. Calibrated, adjustable precision pipets, preferably with disposable plastic tips
3. Glass microscope slides
4. Glass cover slips
5. Deionized water
6. Growth medium
7. FBS

V. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. The sample volumes below are sufficient for 96-wells 100 μ L tests; adjust volumes as needed for the number of wells used in your experiment.

Lucifer Yellow

Briefly centrifuge prior to opening. Add 2 mL distilled water into the vial of Lucifer Yellow. Dissolve completely with vortex mixing and heating at 42°C for 15-20 min. Store unused Lucifer Yellow at -20°C in single use aliquots. Avoid freeze-thaw cycles.

Inhibitor working solution

Briefly centrifuge Inhibitor prior to opening. Dissolve in 50 μ L Inhibitor Diluent to generate Inhibitor stock solution. Then dilute to Inhibitor working solution by adding 1.5 μ L stock solution into 98.5 μ L Diluent. Store unused Inhibitor at -20°C in single use aliquots. Avoid freeze-thaw cycles.

Zymosan working solution

Briefly centrifuge prior to opening. Add 1.5 mL Diluent to each vial of Zymosan and mix well. Boil in a water bath for 10 mins, centrifugation at 2000 $\times$ g 10 min, then wash twice with Wash Buffer (1.5 mL each time), centrifuging at 2,000 $\times$ g for 10 minutes each time. Sonicate washed zymosan for 10 min (3 $\times$ 5 s rest) to disperse clumps. Add 1.5 mL Zymosan Resuspension Resuspend to 1.5 mL sonicated zymosan and mix well. Incubate at 37°C for 30-60 min, vortexing several times during the incubation. Centrifugation at 8000 $\times$ g 10min, then wash 3 times with ice-cold Wash Buffer (3 mL each time), centrifuging at 8,000 $\times$ g for 10 minutes each time. Resuspend in 3 mL ice-cold Diluent. Use immediately or store at -20°C in single use aliquots. Avoid freeze-thaw cycles.

VI. ASSAY PROCEDURE

The sample volumes below are sufficient for 96-wells 100 μL tests; adjust volumes as needed for the number of wells used in your experiment.

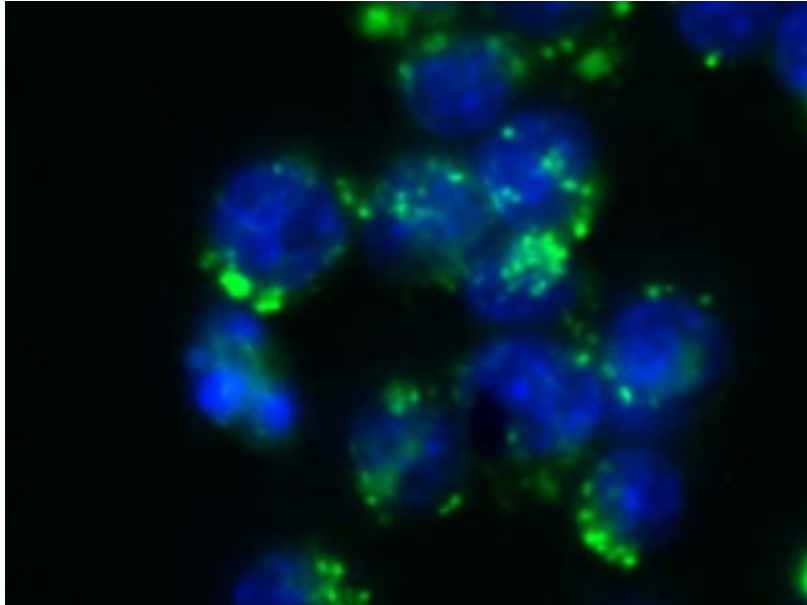
1. Seed macrophages onto coverslips or in well plates. Incubate overnight in a CO_2 incubator.
2. Place the cells on ice and add 50 μL Zymosan working solution to the medium. Incubate the cells on ice for 15 mins.
3. Aspirate the medium, wash 3 times with ice-cold Wash Buffer, 5-10 min each time.
4. Add 90 μL of complete medium. Then add 20 μL of Lucifer Yellow and 10 μL of Inhibitor working solution to the complete medium. Incubate in a CO_2 incubator for 10-20 min.
5. Aspirate the staining solution and wash 3 times with ice-cold Wash Buffer, 5-10 mins each time.
6. Fix with 100 μL Fixation Solution at room temperature for 30 mins (optional, live cells can be observed directly).
7. Observe under a fluorescence microscope (FITC channel).

Fluorescence microscope: Ex/Em = 428/536 nm

Recommended plate: Black wall/clear bottom

Instrument specification(s): FITC filter set

VII. EXAMPLE DATA ANALYSIS AND FIGURES



The fluorescence image of Phagosome Staining in RAW 264.7 cells. Cells were stained at 37 °C for 20min and followed with Hoechst33342 stain. The Diffuse green fluorescent punctate structures were identified as phagosomes.

VIII. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended. Stored at -20°C for 1 year, avoid multiple freeze-thaw cycles.