



E. coli Phagocytosis Assay Kit

Item No. 601370

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
601371	<i>E. coli</i> (FITC/inactivated) Suspension	1 vial/100 tests	4°C
10009322	Cell-Based Assay Buffer Tablet	2 tablets	RT
400292	Trypan Blue (10X)	1 vial/500 µl	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user **must** review the **complete** Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the batch number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A flow cytometer capable of measuring FITC fluorescence (ex/em 485 nm/535 nm)
2. Test tubes or 96-well v-bottom plates as appropriate for your flow cytometer
3. A source of phagocytic cells (such as human PBMCs, mouse bone marrow-derived macrophages, or cell lines)
4. A source of distilled or pure water. *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

About This Assay

Cayman's *E. coli* Phagocytosis Assay Kit employs FITC-labeled, heat-inactivated *E. coli* cells for the measurement of the phagocytic process *in vitro*. The engulfed fluorescent *E. coli* can be detected by flow cytometry. This kit provides enough *E. coli* (FITC/inactivated) Suspension for up to 100 samples.

PRE-ASSAY PREPARATION

NOTE: The E. coli (FITC/inactivated) Suspension is light sensitive. Do not expose to direct intense light.

Reagent Preparation

1. Assay Buffer Preparation

Dissolve each cell-based assay buffer tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. Trypan Blue Quenching Solution Preparation

Prepare a trypan blue quenching solution by diluting the trypan blue stock solution (Item No. 400292) 1:10 in the assay buffer. Mix well to make sure there are no particles or flakes in the solution.

3. *E. coli* (FITC/inactivated) Suspension

Ready to use as supplied.

Flow Cytometry

1. Suspend cells at a concentration of approximately $1-5 \times 10^6$ cells/ml in culture medium.
2. To affect the phagocytic process, cells should be treated as needed for your experiment before adding the *E. coli* (FITC/inactivated) Suspension (Item No. 601371).
3. Place 100 μ l of cells into each well of a 96-well v-bottom plate or each FACS tube.
4. Mix the *E. coli* (FITC/inactivated) Suspension (Item No. 601371) by inversion, then add directly to your pre-warmed culture medium, containing cells, to a final dilution of 1:2 to 1:10.
5. Incubate cells at 37°C for the period of time required for your experiment. Phagocytosis can begin within minutes of *E. coli* addition and continue for hours.
6. To assess the degree of phagocytosis, centrifuge the cells in the plate or tubes at 400 x g for five minutes, remove the supernatant, and resuspend the cells in 200-500 μ l assay buffer. Flow cytometry can be performed immediately.
7. If further staining with antibodies to surface markers or viability is required for your application, maintaining the cells on ice will prevent changes in the FITC fluorescence.
8. To distinguish cells which have phagocytosed the *E. coli* from those simply binding the *E. coli* at the surface, a short (1-2 minute) incubation with 100 μ l of trypan blue quenching solution (prepared on page 7), followed by a wash with assay buffer, will quench surface FITC fluorescence.

Flow Cytometry

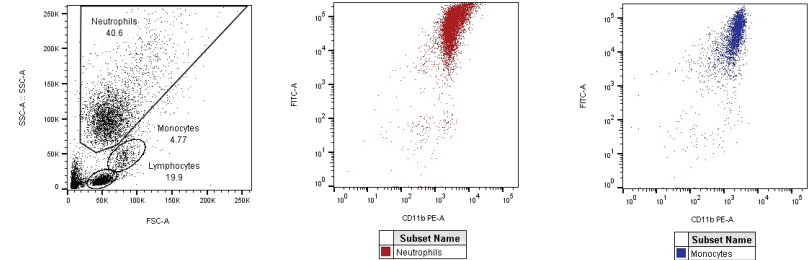


Figure 1. Human peripheral blood leukocytes phagocytose *E. coli* (FITC/inactivated). Fresh peripheral blood leukocytes were isolated and incubated in RPMI medium with a 1:3 dilution of *E. coli* (FITC/inactivated) Suspension for one hour in a test tube. Trypan blue solution was added to quench surface-bound *E. coli*. After pelleting, cells were resuspended in an anti-human CD11b-PE antibody solution and incubated at 4°C for 30 minutes. The cells were washed once with assay buffer and read on a flow cytometer. Using FlowJo® software, CD11b+ neutrophils and monocytes, along with the amount of FITC uptake, are shown in the center and right panels.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Cells do not respond to treatment	<ul style="list-style-type: none"> A. Cells are from a late passage and may have lost the capacity to respond B. Cells are not healthy 	<ul style="list-style-type: none"> A. Use cells at a low passage number B. Use only healthy cells
High background staining in all cells regardless of treatment	<ul style="list-style-type: none"> A. Inadequate washing B. Cells used in the experiment have tendency to attract the <i>E. coli</i> to the membrane 	<ul style="list-style-type: none"> A. Perform washes with assay buffer B. Use Trypan Blue (10X) included in the kit to quench non-specific staining

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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