Vybrant™ Phagocytosis Assay Kit (V-6694)

Quick Facts

Storage upon receipt:
- –20°C
- Protect from light

Number of assays: ~250

Abs/Em: 494/518 nm

Introduction

Our Vybrant™ Phagocytosis Assay Kit (V-6694) is designed to provide a model system for quantitating the effects of drugs or other environmental factors on phagocytic function. Phagocytosis is an important mechanism for nourishment in unicellular organisms and for defense against infection in higher vertebrates. The process of phagocytosis can be observed and quantitated in human polynuclear cells and mouse macrophages by following the internalization of a foreign particle — such as fluorescently labeled immune complexes and bacterial particles. This technique takes advantage of the detectability of the intracellular fluorescence emitted by the engulfed particles, as well as the effective fluorescence quenching of the extracellular probe by trypan blue.[1,2] Our Vybrant Phagocytosis Assay Kit contains fluorescein-labeled *Escherichia coli* (K-12 strain) BioParticles® and a trypan blue solution, as well as step-by-step instructions for performing this phagocytosis assay in a fluorescence microplate reader. Each kit contains sufficient reagents for 250 tests using 96-well microplates. The methodology used with this kit has been developed using an adherent murine macrophage cell line (J774);[2] however, by modifying the cell culture conditions, researchers can adapt this phagocytosis assay to other adherent cell lines.[3]

Storage

Upon receipt, store the kit frozen at -20°C, protected from light. When stored properly, the kit components should remain stable for six months.

Materials

Kit Contents

- **Fluorescein-labeled *Escherichia coli* K-12 BioParticles**, five vials, each containing 5 mg of BioParticles (solid powder).
- **10X concentrated Hanks' balanced salt solution (HBSS)**, five vials, each containing 0.5 mL of buffer.
- **5X concentrated trypan blue**, five vials, each containing 1.0 mL of 1.25 mg/mL trypan blue as a fine suspension in citrate-balanced salt solution, pH 4.4.

Materials Required but Not Provided

- Murine macrophage J774 cells (ATCC®, Rockville, MD) cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 7.5% fetal calf serum, 450 U/mL penicillin and 420 µg/mL streptomycin. Other cell lines may be used as preferred.
- DMEM culture medium
- Hemacytometer (cell-number counter)
- Ion-free water
- Humidified, 37°C and 5% CO_2 ventilated incubator
- Water-bath sonicator
- 96-well microplate with cover and fluorescence microplate reader capable of measuring fluorescence emission at ~520 nm with an excitation at ~480 nm
- Cell viability assay reagents
- Stock solutions of phagocytosis effectors of interest in DMEM

At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform approximately 250 tests.

Phagocytosis Assay Protocol

The following protocol performs ~40 experimental tests as well as appropriate positive and negative control tests on one microplate. The protocol makes use of one vial of each of the three reagent components of the kit (fluorescein-labeled *E. coli* BioParticles, concentrated HBSS and concentrated trypan blue solution).

Preparing the Cells and Fluorescent BioParticles

1.1 Subculture the J774 cells (or other preferred cell type) in DMEM for 3–4 days in advance of performing the assay. Harvest the cells prior to use by scraping them from the surface of the tissue culture dish. Centrifuge the cell suspension and resuspend the cell pellet with the DMEM.

1.2 Determine the cell viability and adjust the cell concentration as appropriate. The cell suspension for use should have >90% viability as determined using any conventional cell-viability assay. Determine the cell concentration using a hemacytometer (cell-number counter)[4] and adjust the final cell concentration to 10^6/mL by adding DMEM to the suspension.

1.3 Prepare the fluorescent *E. coli* BioParticle suspension prior to the experiment as follows. Thaw one vial each of the fluorescent particles and the concentrated HBSS. Pipette the concentrated

HBSS into the fluorescent particle vial and briefly sonicate the suspension. Transfer the suspension into a clean glass tube containing 4.5 mL of deionized water. Sonicate the glass tube until all the fluorescent particles are homogeneously dispersed.

Preparing Experimental and Control Samples

In order to minimize effects of experimental errors, we recommend making measurements on multiple replicates of negative control, positive control and experimental samples. The following protocol makes use of five negative-control wells, five positive-control wells and 40 experimental wells. The numbers of experimental and control wells can be adjusted as required to meet the needs of the particular study.

2.1 Prepare the negative-control wells by adding 150 µL of DMEM to five wells on the microplate.

2.2 Prepare the positive-control and experimental wells by first pipetting 100 µL of the adjusted cell suspension into 45 wells on the microplate.

2.3 Complete the positive-control wells by adding 50 µL of the DMEM to five of the cell-containing wells.

2.4 Complete the experimental wells by adding 50 µL of the phagocytosis effector at desired concentrations in DMEM to the remaining 40 cell-containing wells. In order to minimize effects of experimental errors it is best to make 4–5 replicate tests for each experimental condition.

2.5 Cover the loaded microplate, transfer it to the incubator and incubate for at least 1 hour to allow the cells to adhere to the microplate surface. A longer incubation time may be used if a slow response to the phagocytosis effector is expected.

Adding the Fluorescent Particles and Trypan Blue

3.1 After an appropriate cell incubation time, remove the DMEM solutions from all of the microplate wells by vacuum aspiration.

3.2 Add 100 µL of the prepared fluorescent BioParticle suspension to all the negative control, positive control and experimental wells.

3.3 Cover the microplate and transfer it to the incubator for 2 hours.

3.4 During this incubation period prepare the trypan blue as follows. Thaw a vial of the concentrated trypan blue suspension, mix well and transfer the entire contents to a clean glass tube containing 4 mL of deionized water. Sonicate the glass tube briefly if any precipitate is visible.

3.5 Remove the BioParticle loading suspension from all of the microplate wells by vacuum aspiration.

3.6 Immediately add 100 µL of the prepared trypan blue suspension to all of the wells. Incubate for 1 minute at room temperature after adding the trypan blue.

3.7 Immediately remove the excess trypan blue suspension by vacuum aspiration.

Fluoresence Measurements and Results

4.1 Read the experimental and control wells of the microplate in the fluorescence plate reader using ~480 nm excitation, ~520 nm emission and the appropriate sensitivity settings. To minimize effects of experimental errors, calculate average fluorescence intensity values from groups of 4–5 replicate negative control, positive control and experimental samples.

4.2 Calculate the net phagocytosis and the response to the phagocytosis effector agent. First, subtract the average fluorescence intensity of a group of negative-control wells from that of a group of positive-control wells to yield the ***Net Positive Reading***. This value represents phagocytosis under normal physiological conditions. Second, subtract the average fluorescence intensity of a group of negative-control wells from that of a group of identical experimental wells to obtain the ***Net Experimental Reading***. This value represents phagocytosis in response to the effector. The phagocytosis response to the effector can then be expressed as follows:

$$\% \text{ Effect} = \frac{\text{Net Experimental Reading}}{\text{Net Positive Reading}} \times 100\%$$

References

1. J Immunol Methods 60, 115 (1983); **2.** J Immunol Methods 162, 1 (1993); **3.** J Biol Chem 273, 14813 (1998); **4.** Methods Enzymol 58, 141 (1979).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
V-6694	Vybrant™ Phagocytosis Assay Kit *250 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 ● Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 ● Fax: (541) 344-6504 ● order@probes.com

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Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 ● Fax: (541) 465-4593 ● tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 ● Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 ● Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 ● Fax: +31-71-5241883
eurotech@probes.nl

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