CREATIVE BIOMART®
Assay Kit

Phagocytosis Assay Kit (IgG FITC)

Product Information

Cat.No. Kit-1996

Product Overview

Phagocytosis Assay Kit (IgG FITC) employs latex beads coated with fluorescently-labeled rabbit IgG as a probe for the measurement of the phagocytic process in vitro. The engulfed fluorescent beads can be detected using a fluorescence microscope, allowing kinetic studies of phagocytosis at the single-cell level. In addition, the flow cytometric readout provides the advantage of visualizing perturbations in phagocytosis on the population level and, when combined with antibody staining, of specific cell types within complex populations. This kit provides enough Latex Beads-Rabbit IgG-FITC Complex for up to 750 samples.

Storage

4°C

Shipping

Wet ice

Kit Components

Latex Beads-Rabbit IgG-FITC Complex: 1 vial/150 µl; 4°C
Cell-Based Assay Buffer Tablet: 2 tablets; RT
Trypan Blue (10X): 1 vial/500 µl; RT

Materials Required but Not Supplied

1. A fluorescence microscope or flow cytometer capable of measuring FITC fluorescence (ex/em 485 nm/535 nm)
2. For fluorescence microscopy: appropriate vessels for treating and observing cells (chamber slides or coverslips)
3. For flow cytometry: test tubes or 96 well v-bottom plates as appropriate for your flow cytometer
4. A source of phagocytic cells (such as human PBMCs, mouse bone marrowderived macrophages, or cell lines like RAW 264.7 or THP-1)

Technical Notes

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The Latex Bead-Rabbit IgG-FITC Complex is light sensitive. Do not expose to direct intense light.

Preparation

1. Assay Buffer Preparation
Dissolve each cell-based assay buffer tablet 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 1:10 in the assay buffer. Mix well to make sure there are no particles or flakes in the solution.
3. Latex Beads-Rabbit IgG-FITC Complex Ready to use as supplied. The beads have a 0.1 micron mean particle size.

Assay Protocol

Adherent Cells
1. Plate the cells at a concentration such that they will be less than 80% confluent at treatment and allow to adhere.
2. Add latex beads-rabbit IgG-FITC complex directly to your pre-warmed culture medium to a final dilution of 1:100 to 1:500.
3. Culture cells at 37°C for the period of time required for your experiment. Phagocytosis can begin within minutes of bead addition and continue for hours.
4. For fluorescence microscopy, uptake of beads can be visualized directly in culture with no additional washing steps. However, if staining with live/dead stains or antibodies to surface markers is desired, gentle washing with assay buffer will remove culture medium and unbound beads. Staining can be performed according to your lab's protocols, followed by visualization.
5. For flow cytometry, cells must be removed from the dish in which they are cultured by gentle scraping. Transfer the cells to FACS tubes or 96-well v-bottom plates for further staining or immediate flow cytometry.
6. To distinguish cells which have phagocytosed the beads from those simply binding the beads at the surface, a short (1-2 minute) incubation with trypan blue quenching solution, followed by a wash with assay buffer, will quench surface FITC fluorescence.

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Suspension Cells

1. Suspend cells at a concentration of approximately 1-5 x 10^6 cells/ml in culture medium.
2. Place 100 µl of cells into each well of a 96-well v-bottom plate or each FACS tube.
3. Add latex beads-rabbit IgG-FITC complex directly to your pre-warmed culture medium to a final dilution of 1:100 to 1:500.
4. Incubate cells at 37°C for the period of time required for your experiment. Phagocytosis can begin within minutes of bead addition and continue for hours.
5. 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 µl assay buffer. Flow cytometry can be performed immediately.
6. If further staining with antibodies to surface markers or live/dead dyes is required for your application, maintaining the cells on ice will prevent changes in the FITC fluorescence.
7. To distinguish cells which have phagocytosed the beads from those simply binding the beads at the surface, a short (1-2 minute) incubation with trypan blue quenching solution, followed by a wash with assay buffer, will quench surface FITC fluorescence.

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