PROTOCOL

IncuCyte® pHrodo® Bioparticle® Phagocytosis Assay

For the quantification of phagocytosis of bacterial or yeast pHrodo® Bioparticles®

This protocol provides an overview of the IncuCyte® pHrodo® Bioparticle® Phagocytosis Assay methodology. It is compatible with the IncuCyte® live-cell analysis system using your choice of phagocyte cells, in combination with IncuCyte® pHrodo® Bioparticles® for Phagocytosis reagents.

Required materials

- IncuCyte® pHrodo® Red E. coli Bioparticles® (Essen BioScience Cat# 4615)
  or
- IncuCyte® pHrodo® Green E. coli Bioparticles® (Essen BioScience Cat# 4616)
  or
- IncuCyte® pHrodo® Red Zymosan Bioparticles® (Essen BioScience Cat# 4617)
  or
- IncuCyte® pHrodo® Green Zymosan Bioparticles® (Essen BioScience Cat# 4618)
  or
- IncuCyte® pHrodo® Red S. aureus Bioparticles® (Essen BioScience Cat# 4619)
  or
- IncuCyte® pHrodo® Green S. aureus Bioparticles® (Essen BioScience Cat# 4620)

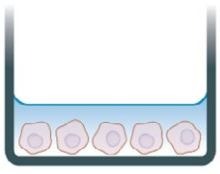
General guidelines

- We recommend medium with low levels of riboflavin to reduce the green fluorescence background. EBM, F12-K, and Eagles MEM have low riboflavin (<0.2 mg/L). DMEM and RPMI have high riboflavin (>0.2 mg/L).
- Following cell seeding, place plates at ambient temperature (15 minutes for adherent cell lines and 45 minutes for non-adherent cell lines) to ensure homogenous cell settling.
- Remove bubbles from all wells by gently squeezing a wash bottle (containing 70-100% ethanol with the inner straw removed) to blow vapor over the surface of each well.
- After placing the plate in the IncuCyte® live-cell analysis system, allow the plate to warm to 37 °C for 30 minutes prior to scanning

NOTE that the mouse macrophage cell line J774A.1 was used to optimize the described conditions, however the methodology can be adapted to accommodate any phagocyte.

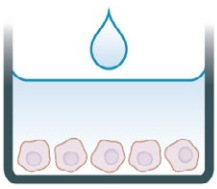
Quick Guide

1 SEED TARGET CELLS



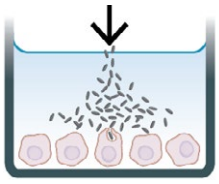
Phagocyte Cell Seeding
Seed phagocytes (50 µL/well, 1 x10^3 to 1 x10^4 cells/well) into the 96-well plate and leave to adhere (2 - 16 h).

2 TREAT CELLS



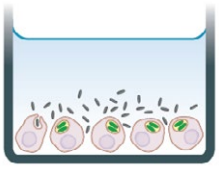
Activator/Inhibitor or Molecular Intervention
Add the desired treatments (25 µL/well) at 4x final assay concentrations.

3 ADD INCUCYTE® PHRODO® BIOPARTICLES® FOR PHAGOCYTOSIS



pHrodo® Bioparticles® Addition
Add your choice of Bioparticle® (e.g. E. coli, S. aureus, Zymosan) to the 96-well plate (approximately 10 µg per well depending on Bioparticle; 25 µL/well at 4x final assay concentrations).

4 LIVE-CELL FLUORESCENT IMAGING



Automated Imaging and Quantitative Analysis
Capture images every 10-30 minutes (20x or 10x) in IncuCyte® system for 2-48 hours. Analyze using integrated software.

IncuCyte® A SARTORIUS BRAND

1 Seed target cells

1.1 Seed phagocytic cells (50 µL per well) at an appropriate density into a 96-well flat bottom plate (Corning, 3595) such that by day 1, the cell confluence is approximately 10 - 20%. The seeding density will need to be optimized for cell type used; however we have found that $1x10^3$ to $1x10^4$ cells per well are reasonable starting points.

NOTE: Phagocyte cell growth can be monitored by recording phase images using the IncuCyte live-cell analysis system and confluence algorithm.

2 Treat cells

2.1 Once the target cells have reached appropriate confluence remove the cell plate from the incubator and add desired treatments. The volumes/dilutions may be varied; however we recommended 25 µL, prepared at 4x final assay concentration.

2.2 Incubate the treatments for the desired duration.

3 Prepare pHrodo Bioparticles and add to cells

3.1 Prepare IncuCyte® pHrodo® Bioparticles® by resuspending to 1 mg/mL in PBS or complete media of choice. Transfer this solution to a glass vial, vortex and sonicate for a minimum of 5 minutes (longer sonication may be required for Zymosan).

NOTE: The formation of a homogeneous suspension may be improved by initial reconstitution in PBS, followed by subsequent dilution in assay media (PBS final assay concentration of 5%).

3.2 After incubation with the treatments, add the IncuCyte® pHrodo® Bioparticles® of your choice to the plate; we recommend 10 µg per well for E. coli/S. aureus or 5 µg for Zymosan.

NOTE: Remove bubbles at the liquid surface by gently squeezing a wash bottle (containing 100% ethanol with the inner straw removed) to blow vapor over the surface of each well.

4 Live-cell imaging

4.1 In the IncuCyte® software, schedule 24 hour repeat scanning for every 15 minutes, 2 images per well, for 2-48 hours (until the fluorescence area and intensity plateaus)

a. Scan on schedule, standard.

b. Channel selection: select "phase" and "red" or "green" (depending on Bioparticle reagent used)

c. Objective: 10x or 20x