SARTORIUS

Product Guide

pHrodo® Bioparticles® for Incucyte® Phagocytosis Assays

Product Information

Presentation, Storage and Stability
pHrodo® Bioparticles® for Incucyte® are supplied as lyophilized solid in sufficient quantity capable of performing 100-200 tests (1 test = 1 well of 96-well microtiter plate). The lyophilized solid should be stored at -20°C and once solubilized the suspension should be stored at +4° C. When stored as described, the lyophilized solid will be stable for at least 6 months and the suspension for at least 1 month.

Background and Intended Use
pHrodo® Bioparticles® for Incucyte® are sterile fluorogenic reagents ideally suited to a simple mix-and-read, real-time live-cell quantification of phagocytosis. The unique pHrodo®-based system exploits the acidic environment of the phagosome to quantify phagocytosis. As pHrodo® Bioparticles® for Incucyte® residing in the neutral extra-cellular solution (pH 7.4) are engulfed by phagocytes and enter the acidic phagosome (pH 4.5–5.5), a substantial increase in fluorescence is observed. Application of pHrodo® Bioparticles® for Incucyte® to non-phagocytic cells yields little or no fluorescent signal. With the Incucyte® integrated analysis software, background fluorescence is minimized. These fully sterilized reagents have been validated for use with the Incucyte® Live-Cell Analysis System and enable real-time evaluation of phagocytic regulation by pharmacological agents as well as genetic and environmental factors.

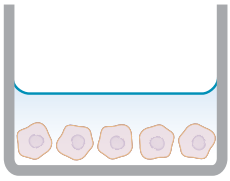
Recommended Use
We recommend that pHrodo® Bioparticles® for Incucyte® are prepared at a stock concentration of 1 mg per mL in full media or PBS. The Bioparticles® may then be diluted for direct addition to cells seeded in a 96-well plate to yield 10 µg per well (for *E. coli* and *S. aureus*) or 5 µg per well (for Zymosan). When used in an Incucyte® Live-Cell Analysis System, we recommend data collection every 15 minutes.

Safety data sheet (SDS) information can be found on our website at **www.sartorius.com**

Product Name	Cat. No.	Amount	Ex. Maxima	Em. Maxima
pHrodo® Red *E. coli* Bioparticles® for Incucyte®	4615	2 mg	560 nm	585 nm
pHrodo® Green *E. coli* Bioparticles® for Incucyte®	4616	2 mg	509 nm	533 nm
pHrodo® Red Zymosan Bioparticles® for Incucyte®	4617	1 mg	560 nm	585 nm
pHrodo® Green Zymosan Bioparticles® for Incucyte®	4618	1 mg	509 nm	533 nm
pHrodo® Red *S. aureus* Bioparticles® for Incucyte®	4619	2 mg	560 nm	585 nm
pHrodo® Green *S. aureus* Bioparticles® for Incucyte®	4620	2 mg	509 nm	533 nm

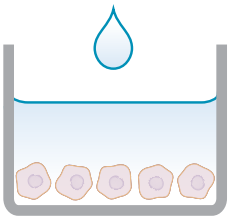
Quick Guide

1. Seed target cells



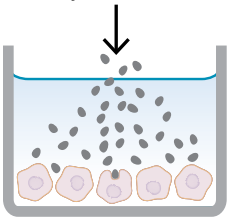
Phagocyte Cell Seeding
Seed phagocytes (50 µL/well, 1×10^3 to 1×10^4 cells/well) into the 96-well plate and leave to adhere (2–16 h).

2. Treat cells



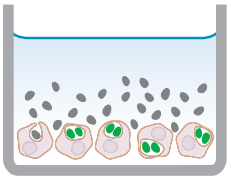
Activator|Inhibitor or Molecular Intervention
Add the desired treatments (25 µL/well) at 4X final assay concentrations.

3. Add pHrodo® Bioparticles® for Incucyte®



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® Live-Cell Analysis System for 2–48 hours. Analyze using integrated software.

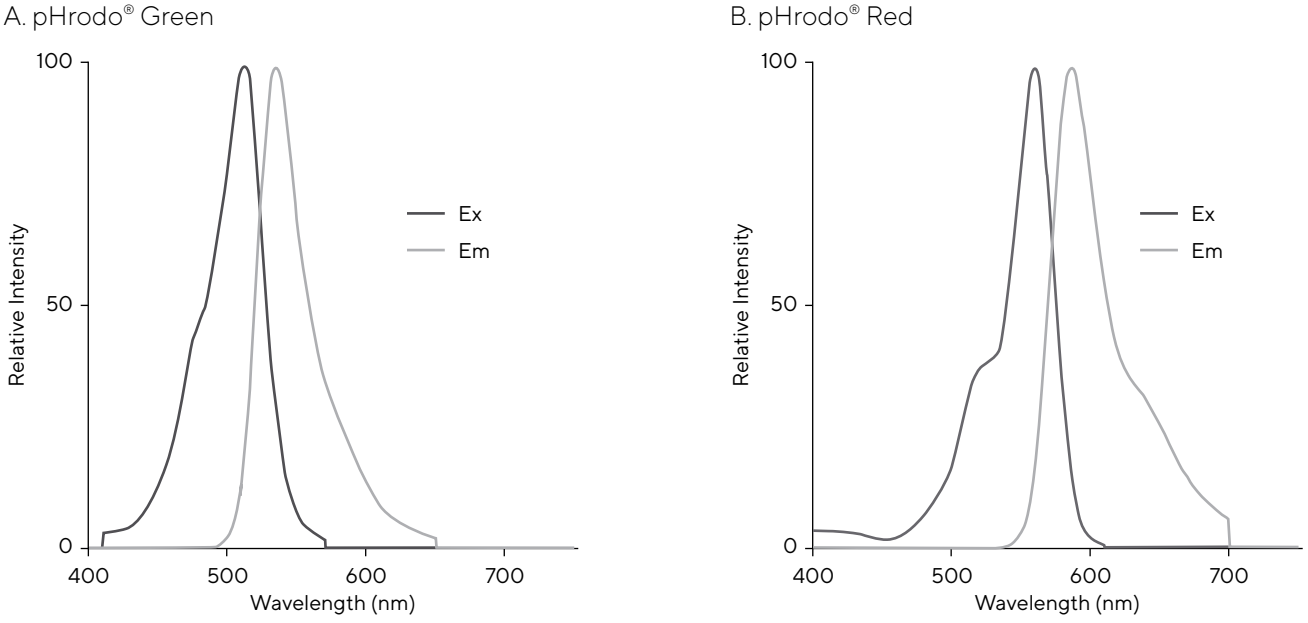


Figure 1
Excitation and Emission Spectra for the (A) pHrodo® Green and (B) pHrodo® Red Fluorophores, Determined in pH 4.0 Buffer

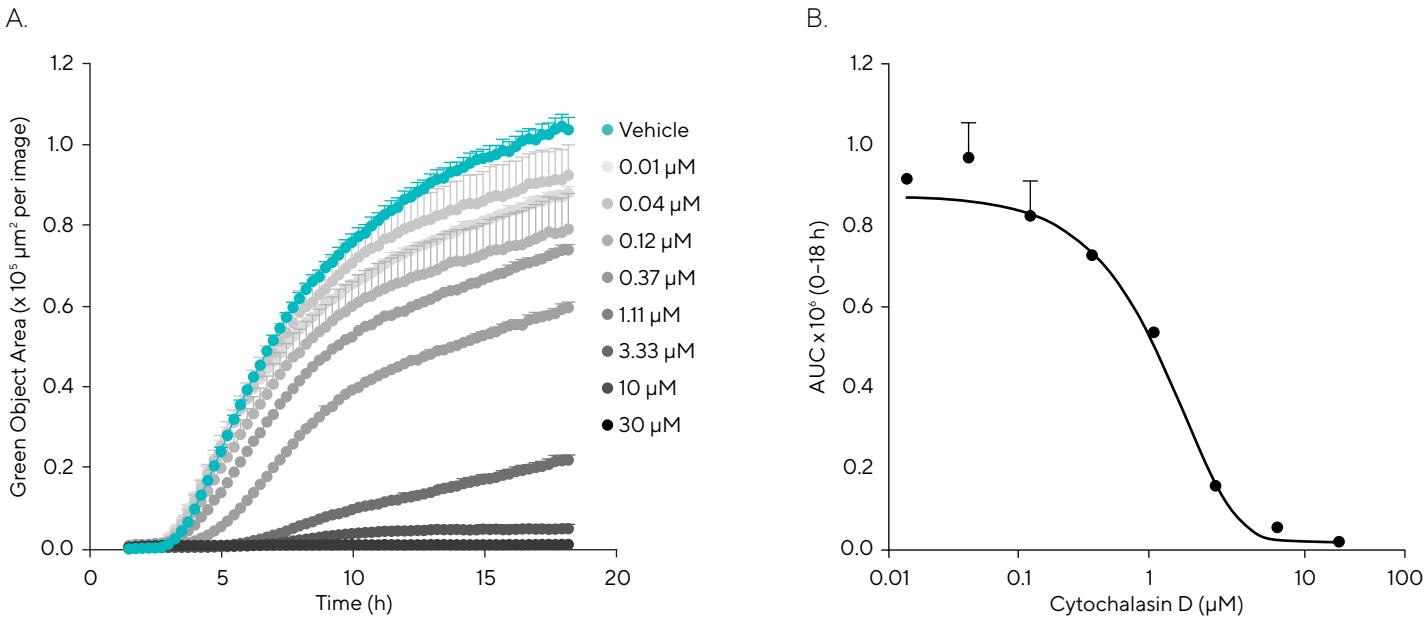


Figure 2

Concentration-Dependent Attenuation of pHrodo® Green E. Coli Bioparticles® Phagocytosis by the Actin Polymerization Inhibitor Cytochalasin D in J774A.1 Murine Macrophages

Note. (A)Time-course of phagocytosis in the absence (open symbols) and increasing concentrations of cytochalasin D (progressively darker gray symbols). Phagocytosis has been quantified as the fluorescence area for each time-point. (B) Concentration response curve to cytochalasin D. Area under the curve (AUC) values have been determined from the time-course shown in panel A (0–18 hours) and are presented as the mean ± SEM, n=3 wells.

Protocols and Procedures

Required Materials

- pHrodo® Red *E. coli* Bioparticles® for Incucyte® (Sartorius Cat. No. 4615)
  or
- pHrodo® Green *E. coli* Bioparticles® for Incucyte® (Sartorius Cat. No. 4616)
  or
- pHrodo® Red Zymosan Bioparticles® for Incucyte® (Sartorius Cat. No. 4617)
  or
- pHrodo® Green Zymosan Bioparticles® for Incucyte® (Sartorius Cat. No. 4618)
  or
- pHrodo® Red *S. aureus* Bioparticles® for Incucyte® (Sartorius Cat. No. 4619)
  or
- pHrodo® Green *S. aureus* Bioparticles® for Incucyte® (Sartorius Cat. No. 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.
- When preparing the pHrodo® Bioparticles®, we recommend using a glass vial to vortex and sonicate the solution for 10–15 min (longer sonification may be required for Zymosan Bioparticles®).
- 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: The mouse macrophage cell line J774A.1 was used to optimize the described conditions; however, the methodology can be adapted to accommodate any phagocyte.

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 Cat. No. 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 1×10^3 to 1×10^4 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 pHrodo® Bioparticles® for Incucyte® 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 10–15 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 pHrodo® Bioparticles® for Incucyte® 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® integrated 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® used)
c. Objective: 10X or 20X

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