

IncuCyte™ pHrodo® Bioparticles® for Phagocytosis

Presentation, storage and stability

IncuCyte™ pHrodo® Bioparticles® for Phagocytosis 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

IncuCyte™ pHrodo® Bioparticles® 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 IncuCyte™ pHrodo® Bioparticles® residing in the neutral extracellular 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 IncuCyte™ pHrodo® Bioparticles to non-phagocytic cells yields little or no fluorescent signal. With the IncuCyte ZOOM® integrated analysis software background fluorescence is minimized. These fully sterilized reagents have been validated for use with the IncuCyte ZOOM® live cell imaging platform and enable real-time evaluation of phagocytic regulation by pharmacological agents as well as genetic and environmental factors.

Recommended use

We recommend that IncuCyte™ pHrodo® Bioparticles® 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 ZOOM® live cell imaging system we recommend data collection every 15 minutes.

Please see the relevant protocol published on our website:
essenbioscience.com/phagoprotocols

Safety data sheet (SDS) information

The SDS can be found on our website
essenbioscience.com/phagocytosis

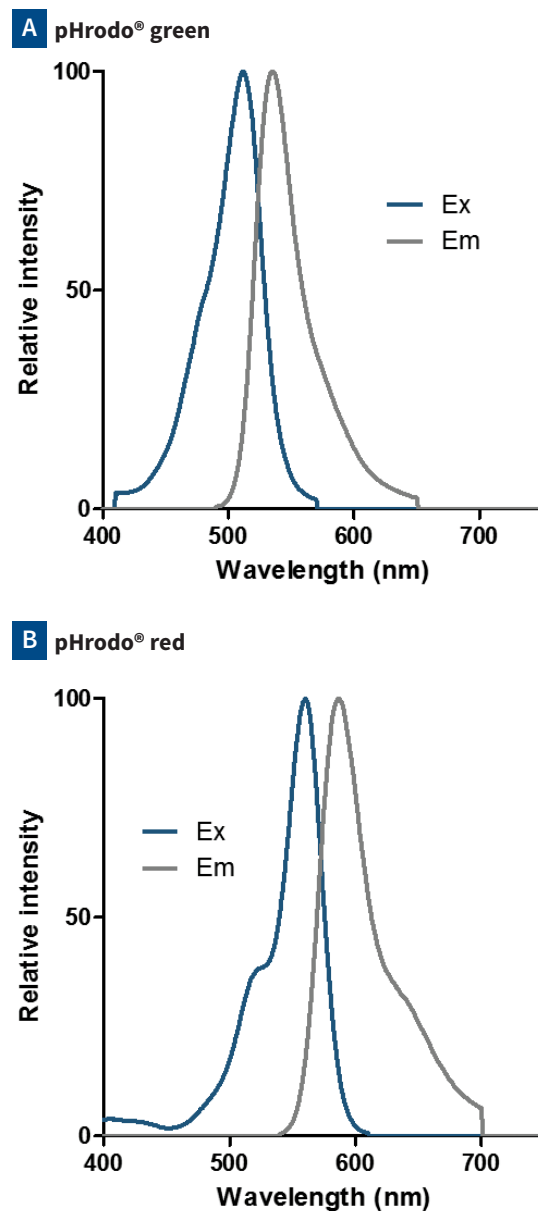
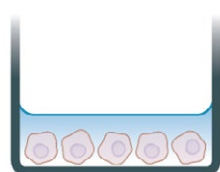


Figure 1. Excitation and emission spectra for the (A) pHrodo® green and (B) pHrodo® red fluorophores, determined in pH 4.0 buffer.

Quick guide

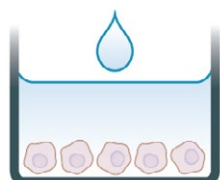
1 SEED TARGET CELLS



Phagocyte Cell Seeding

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

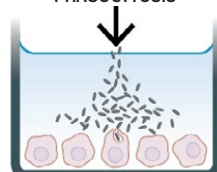
2 TREAT CELLS



Activator/Inhibitor or Molecular Intervention

Add the desired treatments (25 μ L/well) at 4x final assay concentrations.

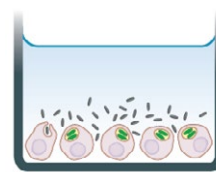
3 ADD INCUCYTE™ PHRODO® BIOPARTICLES® FOR PHAGOCYTOSIS



IncuCyte™ pHrodo® Bioparticles® Addition

Add your choice of Bioparticle® (e.g. *E. coli*, *S. aureus*, Zymosan) to the 96-well plate (approximately 10 μ g per well depending on Bioparticle; 25 μ L/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 ZOOM® for 2-48 hours. Analyze using integrated software.

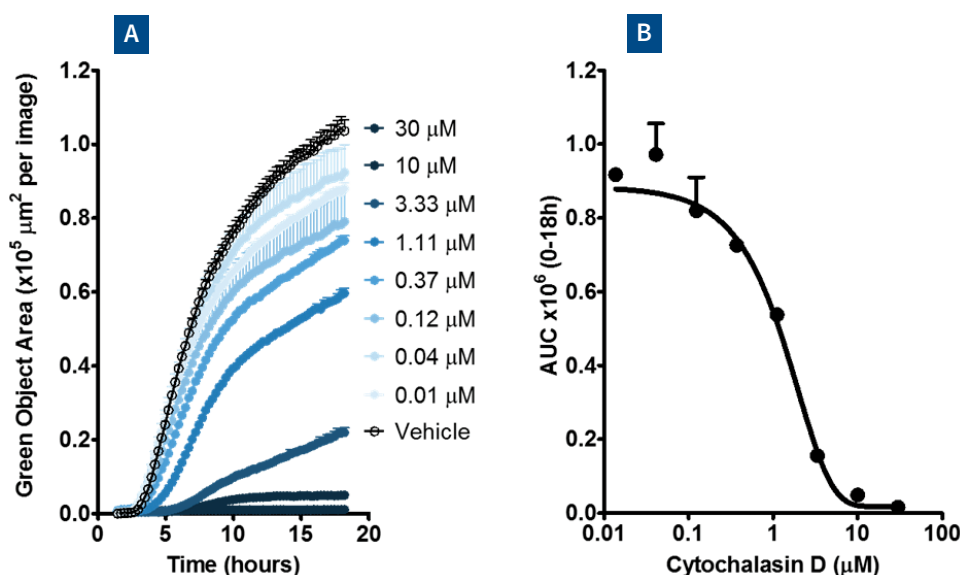


Figure 2. Concentration-dependent attenuation of IncuCyte® pHrodo® Green *E. coli* Bioparticles® phagocytosis by the actin polymerization inhibitor cytochalasin D in J774A.1 murine macrophages.

(A) Time-course of phagocytosis in the absence (open symbols) and increasing concentrations of cytochalasin D (progressively darker blue 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 \pm SEM, n=3 wells.

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

Product	Cat No.	Amount	Ex. maxima	Em. maxima
IncuCyte™ pHrodo® Red <i>E. coli</i> Bioparticles®	4615	2 mg	560 nm	585 nm
IncuCyte™ pHrodo® Green <i>E. coli</i> Bioparticles®	4616	2 mg	509 nm	533 nm
IncuCyte™ pHrodo® Red Zymosan Bioparticles®	4617	1 mg	560 nm	585 nm
IncuCyte™ pHrodo® Green Zymosan Bioparticles®	4618	1 mg	509 nm	533 nm
IncuCyte™ pHrodo® Red <i>S. aureus</i> Bioparticles®	4619	2 mg	560 nm	585 nm
IncuCyte™ pHrodo® Green <i>S. aureus</i> Bioparticles®	4620	2 mg	509 nm	533 nm

Product label licence

RESEARCH Field

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