IncuCyte™ Phagocytosis Assay

Real-time quantitative analysis of phagocytosis

A simple mix-and-read imaging assay for automated quantification of phagocytosis over time in living cells, within your incubator. The assay provides real-time visualization and analysis of the internalization of bioparticles using pH sensitive-conjugated probes, and is ideal for monitoring phagocytosis of bacterial gram positive, gram negative or yeast-derived pathogens by immune cells.

Key Advantages of the IncuCyte™ Phagocytosis Assay
- Validation of phagocytosis using time-lapse imaging. (Figure 1)
- Automated analysis and quantification. (Figure 2)
- Mix-and-read 96/384-well format, no fixing, no quenching, no lifting. (Figure 3)
- Highly sensitive, low background, low cell numbers. (Figures 4 & 5)
- Sterile IncuCyte™ pHrodo® Bioparticles® enable long-term measurements (0 to >48 hours) in real time.

Figure 1. Validation of phagocytosis using time-lapse imaging. Time-lapse visualization of J774A.1 mouse macrophages phagocytosing IncuCyte™ pHrodo® Green *E. coli* Bioparticles® over 4 hours. Images verify the presence of fluorescent punctate (phagosomal) labelled structures in the cytosol but not in the nucleus.

Measure green fluorescence; IncuCyte™ pHrodo Green *E. coli* Bioparticles®

Measure red fluorescence; IncuCyte™ pHrodo Red *S. aureus* Bioparticles®

Figure 2. Quantification of phagocytosis using fluorescence segmentation. IncuCyte™ software enables accurate segmentation of the fluorescence image (pink mask) and minimizes the impact of background fluorescence.
**Figure 3.** IncuCyte™ phagocytosis assay quick start guide.

1. **SEED TARGET CELLS**
   - **Phagocyte Cell Seeding**
     - Seed phagocytes (50 µL/well, 1 x 10³ to 1 x 10⁴ 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**
   - **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 ZOOM® for 2-48 hours. Analyze using integrated software.

**Figure 4.** Quantification of phagocytosis is cell number dependent. J774A.1 mouse macrophages phagocytosing IncuCyte™ pHrodo® Green S. aureus Bioparticles® (96-well format). Note high signal: background with as low as 3K cells per well.

**Figure 5.** Inhibition of phagocytosis by Cytochalasin D, Latrunculin A, Nocodazole. J774A.1 mouse macrophages phagocytosing IncuCyte™ pHrodo® Green E. coli Bioparticles® in the presence of inhibitors.
**Assay Concept**

1. Macrophages (e.g. J774A.1 mouse macrophages) or other phagocytic cells are seeded in 96- or 384-well micro-titer plates.
2. Phagocytosis is measured in real-time by adding mix-and-read sterile IncuCyte™ pHrodo® Bioparticles®.
3. An increase in cellular fluorescence indicates the internalization of Bioparticles® into the phagosome. IncuCyte™ automated image analysis enables quantitation of phagocytosis over time.

**IncuCyte™ Phagocytosis Assay vs. Other Common Approaches**

Common methods used to assess phagocytosis are often end-point (e.g. High Content Analysis (HCA)), require cell lifting (e.g., flow cytometry) or washing/quenching of the labelled pathogen (e.g. fluorescein labelled).

<table>
<thead>
<tr>
<th>Reader</th>
<th>IncuCyte™ ZOOM</th>
<th>Plate reader</th>
<th>Flow cytometry</th>
<th>HCA</th>
<th>ELISA</th>
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<tbody>
<tr>
<td>Real-time cell visualization</td>
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**RELATED APPLICATIONS**

**Chemotactic Cell Migration and Invasion Using the IncuCyte ZOOM® System**

- Real-time imaging and quantitation of chemotactic cell migration and invasion for label-free or fluorescently labeled cells from within your incubator.
- See everything with high definition images including morphological changes, cell-cell interactions and collective cell migration and invasion.
- Highly reproducible kinetic read-outs compatible with detailed mechanistic studies and/or 96-well screening and profiling.

Learn more at essenbioscience.com/chemotaxis
### Ordering information

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<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. No.</th>
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Learn more at essenbioscience.com/phagocytosis/

### Other Key Applications Using the IncuCyte ZOOM® Live-Cell Imaging System

- **3D-Spheroids**
- **Angiogenesis**
- **Apoptosis**
- **Cell Culture QC**
- **Chemotaxis**
- **Migration & Invasion**
- **Cytotoxicity**
- **Dilution Cloning**
- **Immune Cell Killing, Clustering & Proliferation**
- **Neurite Dynamics - Label-Free**
- **Neuronal Co-Culture - Fluorescence**
- **Phagocytosis**
- **Proliferation - Cell Count**
- **Proliferation - Confluence**
- **Reporter Gene**
- **Scratch Wound Migration & Invasion**
- **Stem Cell Monitoring & Reprogramming**
- **Transfection Efficiency**

Learn more at essenbioscience.com/applications/