

# Holotomography-Monitored Phagocytosis Assay of M1 Macrophages

PromoCell

## Application Note

### Background

Macrophages are tissue-resident professional phagocytes and antigen-presenting cells (APC) that are derived from circulating peripheral blood monocytes. They perform important active and regulatory functions in both adaptive and innate immunity, e.g. by clearing bacterial infections from open wounds.

Macrophages are able to recognize bacteria by activating special cell surface receptors bind to various components of bacterial cell walls. A bacterium is engulfed by the macrophage's cell membrane and the resulting vesicle constricted to form a so-called phagosome that is moved into the macrophage's cytoplasm. There it undergoes a maturation process and enters the phagolysosomal pathway, eventually leading to its degradation and complete clearance. The phagosome fuses with a lysosome, which is an acidic intracellular vesicle containing large amounts of hydro-

lytic enzymes, form a cytoplasmic body called a phagolysosome. It therefore also has an acidic pH, thus providing an optimal environment for enzymatic degradation of bacterial pathogens.

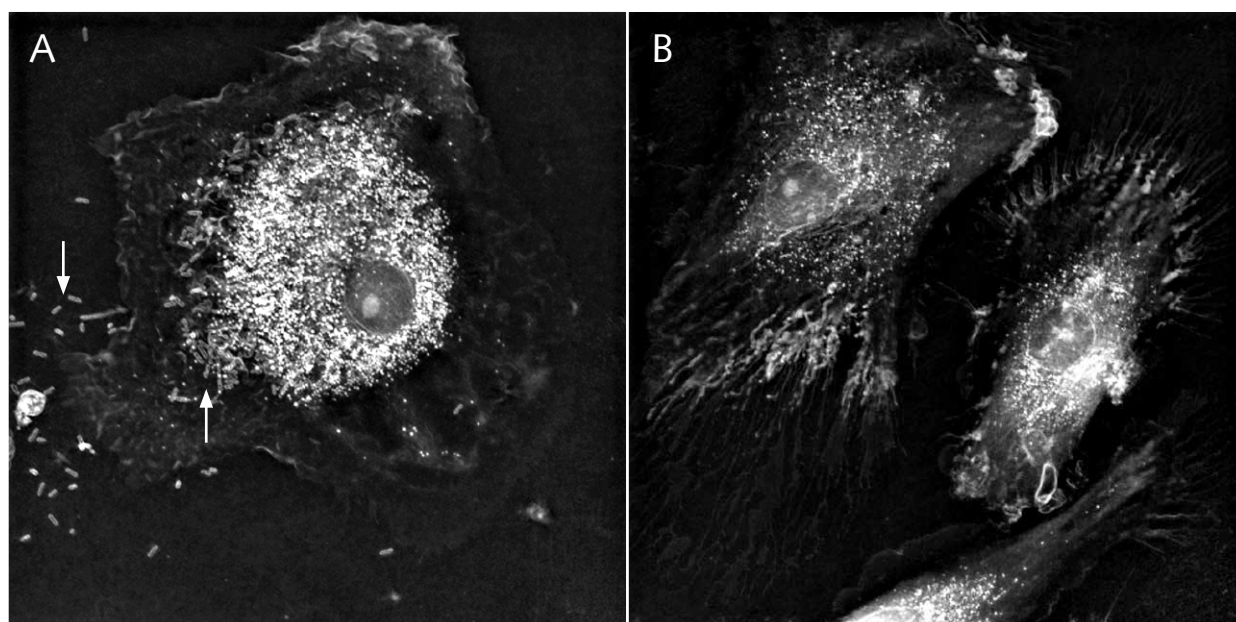
### PromoCell's Phagocytosis Assay Kit (*E. coli*)

To test the viability of macrophages *in vitro*, a phagocytosis assay is an excellent way to determine whether they are working properly. Phagocytosis assays often use *E. coli* and other bacterial strains as the pathogens. PromoCell's Phagocytosis Assay Kit (*E. coli*) uses heat-killed, red or green fluorescently labeled *E. coli* particles for fast, accurate detection and quantification of *in vitro* phagocytosis. The kit provides a robust screening system for all activators and/or inhibitors of phagocytosis and toll-like receptor (TLR) ligands. Analysis can be performed using various microscopy techniques, spectrophotometry, or flow cytometry.

### Nanolive's Cell Explorer for live cell holotomography

Nanolive imaging is a versatile observational tool. It is a technique for measuring a three-dimensional refractive index tomogram of a microscopic specimen that eliminates the need to use fluorescent labels or modify the physiological environment of the cells *in vitro*. Cellular processes can be directly monitored and analyzed. Rotational scanning allows 3D reconstructions while providing noise robustness and a resolution far greater than is possible with standard light microscopy.

The 3D Cell Explorer from NANOLIVE uses a globally unique technology that has been patented in the U.S. (no. 8,937,722) and European Union (publication no. WO/2011/121523).



**Fig. 1: Cryopreserved human M1 macrophages (GM-CSF).** Macrophages were plated with fibronectin and monitored in the Cell Explorer workstation (5% CO<sub>2</sub>). The experiments were performed during 24 hours, with a frame rate of 6Hz and a field of view of 85 x 85 x 30 μm. Watch the live footage [here](#). **A**, M1 macrophage phagocytoses *E. coli* (inside and outside of the cell). **B**, [Macrophage movement](#) cultured in M1-Macrophage Generation Medium DXF on glass-bottom cell culture vessels.

*Use aseptic techniques and a laminar flow bench.*

## Phagocytosis Assay Kit (*E. coli*) Protocol

### I. Materials

- Proliferating culture of human macrophages in good condition (C-12914 or C-12916)
- M1- or M2-Macrophage Generation Medium DXF (C-28055 or C-28056)
- Phagocytosis Assay Kit (*E. coli*) (PK-CA577-K964)
- 3D Cell Explorer workstation and its controlling software STEVE (<https://nanolive.ch/cx/>)
- Glass-bottom dishes or cell culture vessels compatible with the 3D Cell Explorer
- Centrifuge

### II. Storage and Reagent Preparation

- Store the entire kit at 4°C, protected from light.
- Phagocytosis Assay Buffer: Upon arrival, combine one entire vial of buffer additive with one bottle of phagocytosis assay buffer and mix well.
- Green *E. coli*: Before use, acclimate the suspension to room temperature and vortex gently for 5 seconds.
- Quenching Solution: Dilute the content of the vial into 4.5 ml of 1x phagocytosis assay buffer

### III. Assay

#### 1. Preparation of control and experimental wells

Subculture cells capable of phagocytosis in appropriate medium. Please read more in our [Cryopreserved Macrophage Application Note](#).

#### 2. Day -1

Determine viability of cells (e.g. using the colorimetric WST-8, the fluorometric resazurin or the bioluminescent ATP-based assay) and re-suspend cells to a concentration of 1 - 5 x 10<sup>6</sup> viable cells/ml. Aliquot 100 µl of the cell culture per well omitting the negative control wells and incubate the plate overnight at 37°C, 5% CO<sub>2</sub>.

#### 3. Day 0

Change the media and proceed to the phagocytosis effector assay. Your experiment should always include parallel negative, positive and experimental wells.

#### 4. Additional Phagocytosis Effector Assay

Add 100 µl of cell culture media containing your effector of interest (not provided in the kit) at the desired concentration (e.g. 20 µM Cytochalasin D; see Related Products) to each of the experimental wells. Aliquot 100 µl of media to each of the positive and 200 µl media in each of the negative control wells respectively. Incubate for 1 hour at 37°C, 5% CO<sub>2</sub>.

#### 5. Phagocytosis of *E.coli*

Add 5 µl of *E. coli* slurry to all the wells. Immediately transfer the plate back to the incubator for 2 – 3 hours. The incubation time may be adjusted according to your protocol.

## Phagocytosis Assay Protocol

*Use aseptic techniques and a laminar flow bench.*

#### 6. *E. coli* Standard Curve

Add 0, 1, 2, 3 and 4  $\mu$ l of Green *E. coli* slurry to the cell culture vessel. Adjust the volume to 100  $\mu$ l with phagocytosis assay buffer. Mix well. Immediately measure and plot the standard curve.

#### 7. Detection

Cell activity can be analyzed by holotomography, FACS or other microscopic techniques.

### IV. Live cell analysis with the 3D Cell Explorer

#### 1. Preparation

Allow proliferating culture of human macrophages to grow on glass-bottom dishes compatible with the 3D Cell Explorer.

#### 2. Live observation of phagocytosis of M1 macrophages

Prepare the 3D Cell Explorer workstation and its controlling software STEVE. Image frame rate 6Hz, experimental setup, STEVE, FOV of 85 x 85 x 30  $\mu$ m, air objective, 60x.

#### 3. Possible parameters for analyzing and determining the viability of macrophages

- Percentage of macrophages containing bacteria
- Average number of intracellular bacteria per macrophage
- Percentage of macrophages with attached bacteria
- Average number of attached bacteria per macrophage

*Phagocytosis  
Assay  
Protocol*

*Holotomography  
Cell Analysis  
Protocol*

### Products

Product	Size	Catalog Number
Human M1 Macrophages (GM-CSF), monocyte-derived, single donor	1.5 Mio/ 5 Mio	C-12914/ C-12916
Human M2 Macrophages (M-CSF), monocyte-derived, single donor	1.5 Mio/ 5 Mio	C-12915/ C-12917
Fibronectin Solution, human (1 mg/ml)	5 ml	C-43060
M1-Macrophage Generation Medium DXF (incl. GM-CSF)	250 ml	C-28055
M2-Macrophage Generation Medium DXF (incl. M-CSF)	250 ml	C-28056
Phagocytosis Assay Kit ( <i>E. coli</i> ) green/red	100 assays/100 assays	PK-CA577-K963/ PK-CA577-K964
3D Cell Explorer	Holotomographic microscope	<a href="http://nanolive.ch/cx">nanolive.ch/cx</a>

### Related Products

Cell Viability Assays	Size	Catalog Number
Colorimetric Cell Viability Kit I (WST-8)	500 assays	PK-CA705-CK04
Fluorometric Cell Viability Kit I (resazurin)	5 ml	PK-CA707-30025-0
Bioluminescent Cell Viability Kit I (ATP)	200 assays	PK-CA577-K254-200



## Related Products

Cytokines & Activation Factors	Size	Catalog Number
Dexamethasone	1 g	PK-CA577-1042-1G
IFN- $\gamma$ ( <i>E. coli</i> ) / IFN- $\gamma$ (plant)	100 $\mu$ g*	C-60724 / C-60724A
M-CSF ( <i>E. coli</i> ) / M-CSF (plant)	10 $\mu$ g*	C-60442 / C-60442A
GM-CSF ( <i>E. coli</i> ) / GM-CSF (plant)	20 $\mu$ g* / 10 $\mu$ g*	C-60424 / C-60420A
Cryo-SFM	30 ml	C-29910
IL-1 beta ( <i>E. coli</i> ) / IL-1 beta (HEK)	10 $\mu$ g* / 10 $\mu$ g*	C-61120 / C-61121
IL-4 ( <i>E. coli</i> ) / IL-4 (Plant) / IL-4 (HEK)	20 $\mu$ g* / 10 $\mu$ g* / 10 $\mu$ g*	C-61421 / C-61420A / C-61452
IL-6 ( <i>E. coli</i> ) / IL-6 (CHO) / IL-6 (HEK)	20 $\mu$ g* / 10 $\mu$ g* / 10 $\mu$ g*	C-61625 / C-61640 / C-61642
IL-10 ( <i>E. coli</i> ) / IL-10 (HEK)	10 $\mu$ g*	C-62012 / C-62014
IL-12 (CHO) / IL-12 (HEK)	10 $\mu$ g*	C-62213 / C-62212
IL-13 ( <i>E. coli</i> )	10 $\mu$ g*	C-62312
MCP-1 (CCL2)	10 $\mu$ g*	C-64030
MIP-1 alpha (CCL3) / MIP-3 alpha (CCL20)	20 $\mu$ g* / 20 $\mu$ g*	C-65833 / C-65866
TGF- $\beta$ 1 ( <i>E. coli</i> ) / TGF- $\beta$ 1 (CHO) / TGF- $\beta$ 1 (HEK)	10 $\mu$ g*	C-63500 / C-63503 / C-63499

\*Bulk sizes (100/250/500 and 1000  $\mu$ g) are available on request.  
More cytokines/chemokines/growth factors are available at [www.promocell.com](http://www.promocell.com).

Cell-Based Assays	Size	Catalog Number
Cell Migration/Chemotaxis Assay Kit (8 $\mu$ m)	12 assays (24-well)/ 100 assays (96-well)	PK-CA577-K909/ PK-CA577-K906
Cell Migration/Chemotaxis Assay Kit (5 $\mu$ m)	12 assays (24-well)/ 100 assays (96-well)	PK-CA577-K910/ PK-CA577-K907
Cell Migration/Chemotaxis Assay Kit (3 $\mu$ m)	12 assays (24-well)/ 100 assays (96-well)	PK-CA577-K911/ PK-CA577-K908
Lysosomal Intracellular Activity Assay Kit	50 assays	PK-CA577-K448
Lysosomal Cytotoxicity Dual Staining Kit	50 assays	PK-CA577 K458
Phagocytosis Assay Kit (Zymosan) green/red	100 assays/100 assays	PK-CA577-K397/ PK-CA577-K398

## References

- 1 Ovchinnikov, D.A., et al., *Macrophages in the embryo and beyond: much more than just giant phagocytes*. *Genesis*, 2008 46(9): p. 447–62. doi:10.1002/dvg.20417.
- 2 Kloc, *Macrophages Origin, Functions and Biointervention*. Book, Springer, 2017, ISBN 978-3-319-54090-0

This is a collaboration of:

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