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High Throughput Phagocytosis assay

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We use this protocol and it's working

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Abstract

Protocol for a longitudinal phagocytosis assay using PhroRed zymosan particles (ThermoFisher) and the Incucyte live-cell analysis system (Sartorius).

Preparing cells for assay

1 Differentiate microglia as described in dx.doi.org/10.17504/protocols.io.dm6gpdrp5gzp/v1

2 Plate microglia-like cells in a PhenoPlate 96-well (Revvity) at 150,000 cells/mL, 100 µL per well in X-Vivo 15 (Lonza) supplemented with 1% Glutamax, 0.5% Pen/Strep and 100ng/mL MCSF.

3 Wait for one week for microglia to mature

Phagocytosis assay

4 Incubate cells with [M] 100 ug/mL pHrodo Red Zymosan BioParticles Conjugate for Phagocytosis (ThermoFisher) in supplemented X-vivo.

5 Immediately place the PhenoPlate in Incucyte Live-Cell analysis system (Sartorius).

6 Set the analysis system to acquire images from each well of the plate at 560/585 nm and in Phase contrast. For a full plate this takes ~30 minutes, for a longitudinal assay images can be acquired every 45 minutes.

7 For a longitudinal assay, acquire images for 5 to 8 hours after incubation with PhrodoRed particles to observe the progress of uptake by microglia.

> Note
>
> Adding one extra acquisition step before incubating the cells with the probe can be useful. The plate can be removed in between two acquisition steps to treat with probes/drugs for longitudinal assays.

8 For analysis, the inbuilt pipelines of the Incucyte allow to measure the intensity, area and puncta count of the PhrodoRed signal, as well as confluence % based on Phase Contrast, making it possible to visualise the evolution of phagocytosis in cells.