Phagocytosis Assay Protocol

Product Code: JIPSC002072

Cell line Name: CLYBL 6-TF-iMG KI1/KI2

Parental Line: KOLF2.1J

Protocols

The standard protocol for thawing, propagating, and freezing KOLF2.1J lines is available on the JAX iPSC webpage (jax.org/ipsc). The protocol for differentiating KOLF2.1J CLYBL 6-TF-iMG KI1/KI2 into microglia can be found here: https://www.jax.org/jax-mice-and-services/ipsc/cells-collection/JIPSC002072

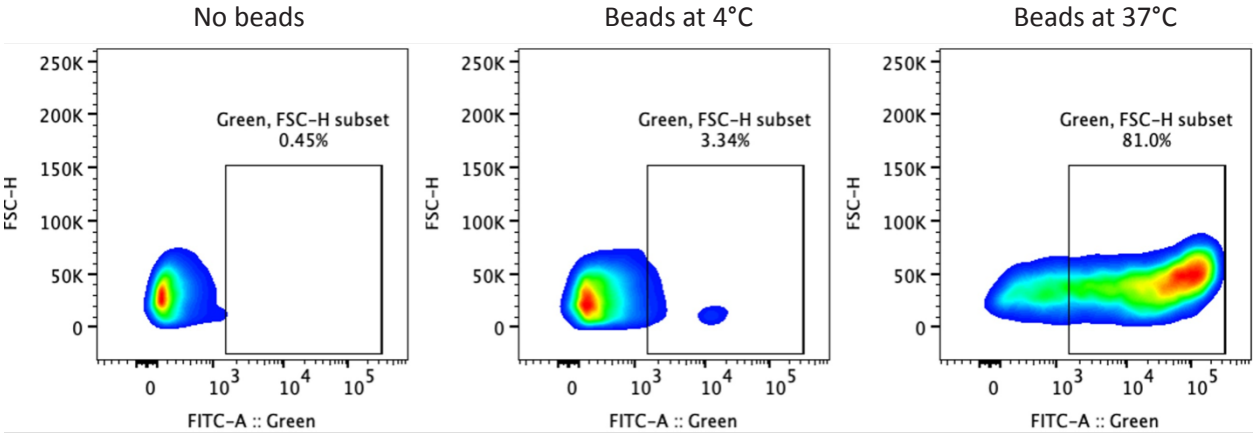
The following **Phagocytosis Assay Protocol** is a modified version of the method described by *Erra-Diaz et al. (2023) in Cell Reports*[1].

Flow Cytometry-Based Analysis

1. Follow the coating and differentiation instructions outlined in the accompanying protocol, culturing microglia-like cells to maturation over approximately 9-10 days.
2. Incubate mature iMicroglia with 100 µg/mL fluorescently labeled zymosan beads (pHrodo™ Green *E. coli* BioParticles™ Conjugate for Phagocytosis, ThermoFisher Scientific, #P35366) for 2.5 hours. After incubation, process the cells for quantification of phagocytic activity by flow cytometry.
    a. Use wells containing only cells (without beads) as negative controls.
    b. To assess nonspecific binding, incubate plates containing 100 µg/mL zymosan beads and cells at 4°C for 2.5 hours

Dissociation of iMicroglia for Flow Cytometry:

3. Remove iMicroglia culture media and gently wash the cells with 1X PBS.
4. Incubate the cells with TrypLE™ Express Enzyme (Thermo Fisher Scientific, #12604013) for 7 minutes at 37°C.
5. Add Advanced DMEM media (Thermo Fisher Scientific, #12491015) to neutralize TrypLE™ and collect floating cells.
6. Centrifuge the collected cells at 1600 RPM for 5 minutes.
7. Resuspend the cell pellet in 1X PBS and repeat centrifugation (1600 RPM, 5 minutes).
8. Resuspend the final cell pellet in FACS buffer (1X PBS with 2% Fetal Bovine Serum, Thermo Fisher Scientific, #A5256801) and proceed with the Flow Cytometry analysis.



iMicroglia without zymosan (negative control) and with fluorescently labeled zymosan beads incubated at 4°C (nonspecific binding control) and 37°C (bead uptake condition).

Fluorescent Microscopy-Based Analysis

For confocal imaging analysis, differentiate cells on coated glass-bottom plates (see coating instructions below) to enhance adherence and imaging quality.

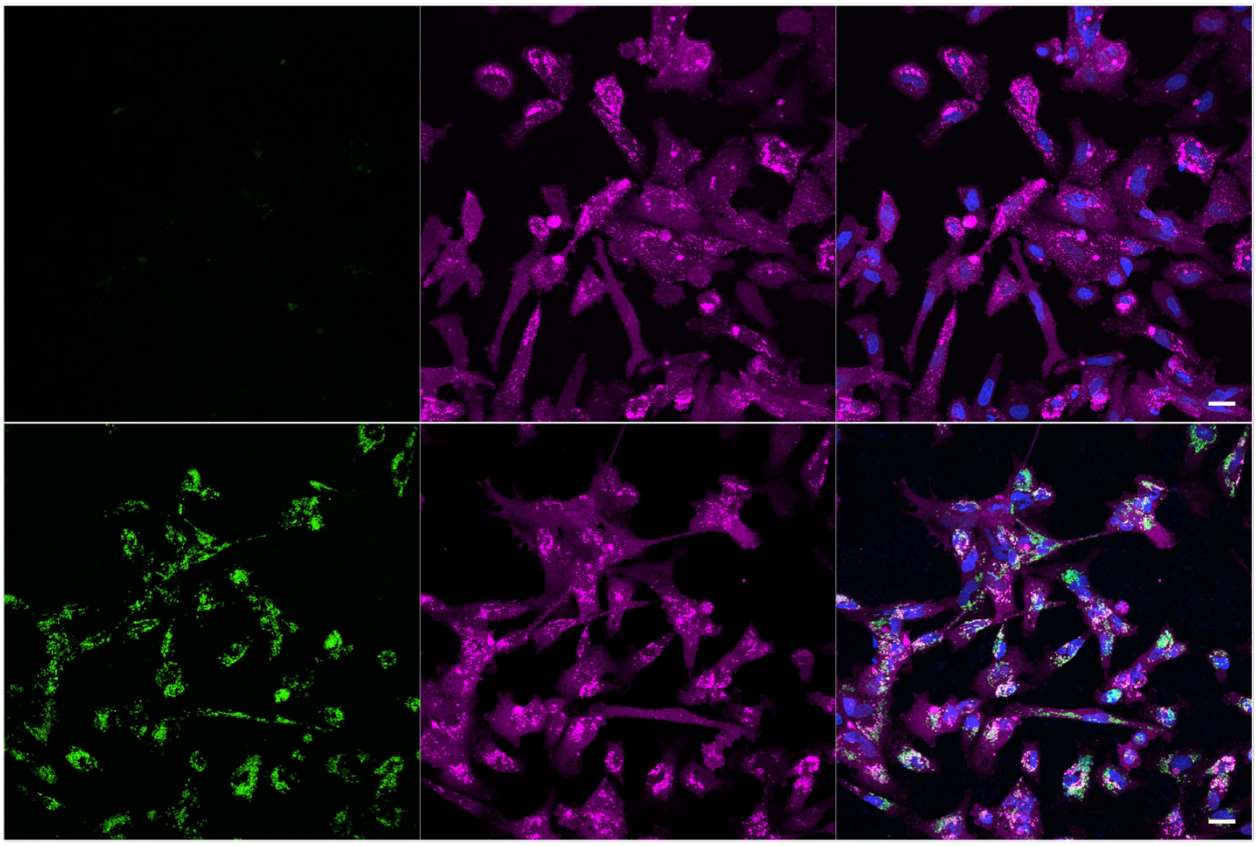
For live-cell imaging, use mCherry-expressing iMicroglia or phalloidin staining for visualization. Maintain proper media and temperature control throughout imaging to preserve cell viability.

1. Incubate cells with fluorescently labeled zymosan beads (100 µg/mL) for 1 hour, as described above.
2. Stain nuclei by treating iMicroglia with 2 µg/mL Hoechst (Invitrogen, #H21592) for 20 minutes at 37°C.
3. Remove the media and gently wash cells once with fresh media to eliminate excess stain and beads.
4. Replace with fresh media before initiating image acquisition to maintain optimal cell conditions.

Glass Plate Coating Instructions

Use glass-bottom plates pre-coated with Poly-D-Lysine (Corning, #356414) or CellView culture dishes (Greiner Bio-One, #627860) for imaging. To enhance cell adherence, apply a double coating with Poly-D-Lysine (Thermo Fisher Scientific, #A3890401) and laminin 521 (BioLamina, #LN521).

1. If not using pre-coated plates, coat the slides with Poly-D-Lysine following the instructions in the differentiation protocol on the JAX iPSC webpage (jax.org/ipsc).
2. Apply 5 µg of laminin 521 and incubate for 18 hours at 4°C to optimize cellular attachment and imaging conditions.



Confocal images of iMicroglia expressing mCherry, without (top) or with (bottom) fluorescent-labeled zymosan beads (green). Nuclei stained with Hoechst (blue). Magnification: 40X.

References

1. Erra Diaz F, Mazzitelli I, Bleichmar L, Melucci C, Thibodeau A, Dalotto Moreno T, Marches R, Rabinovich GA, Ucar D, Geffner J. Concomitant inhibition of PPARγ and mTORC1 induces the differentiation of human monocytes into highly immunogenic dendritic cells. Cell Rep. 2023 Mar 28;42(3):112156. doi: 10.1016/j.celrep.2023.112156. Epub 2023 Feb 26. PMID: 36842088.
2. Oliveira NAJ et al. 2025 *bioRxiv*

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