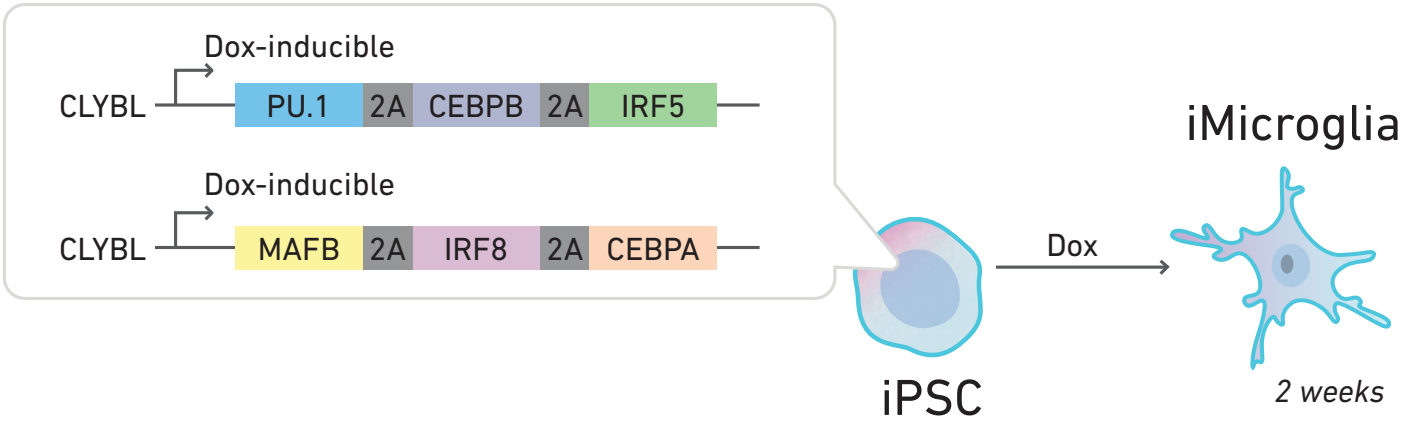
iMicroglia Differentiation Protocol for KOLF2.1J CLYBL 6-TF-iMG KI1/KI2

Product Code: JIPSC002072

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

Parental Line: KOLF2.1J

Overview



Protocols

The standard protocol for thawing, propagating, and freezing KOLF2.1J lines is available on the JAX iPSC webpage (jax.org/ipsc).

Differentiation protocol

Protocol Modified from Dräger et al. 2021, Nature Neuroscience.
Oliveira et al. 2025 bioRxiv

1. Before differentiation, iPS cells must be adapted to mTeSR™ Plus Medium (*STEMCELL Technologies, #100-0276*). Grow cells to at least 50-70% confluency and culture for at least one to two passages in mTeSRTM Plus Medium, changing media every two days as necessary.
2. If cells were cultured in StemFlex™ Medium on a Synthemax®-coated plate, switch to mTeSR™ Plus Medium 16-24 hours after passaging. Proceed with differentiation according to the instructions below.

Day 0

1. Dissociate cells from the 6-well plate by adding 1 mL of Accutase (Thermo Fisher Scientific, #00-4555-56) per well and incubate at 37°C for 7-10 minutes to achieve a single-cell suspension. Then, plate the cells in Day 0 Medium on **double-coated plates**.
2. To generate double-coated plates, follow the instructions below:

    a. **Poly-D-Lysine Coating**

        i. In a 50 mL conical tube, mix 22.5 mL H_2O with 2.2 mL Borate buffer (Thermo Fisher Scientific, #28341). Filter H_2O and Borate buffer solution.

        ii. Add 1.25 mL Poly-D-Lysine (0.1 mg/mL, Thermo Fisher Scientific, #A3890401) to filtered solution and mix well.

        iii. Add 2 mL to each well of a 6-well plate.

        iv. Incubate for two hours at 37°C or overnight at 37°C (If left overnight, consider wrapping plate in Parafilm before placing into the incubator).

    b. **Laminin 521 Coating**

    Dilute the Laminin 521 stock solution in 1X DPBS (Ca^{2+}/Mg^{2+}) to achieve a final coating concentration of 5 µg/mL. Then, apply the diluted solution to the desired cell culture vessel.

    For a 6-well plate:

        i. Mix 950 µL of 1XDPBS with 50 µL Laminin 521 stock (BioLamina, #LN521) to achieve a final concentration of 5 µg/mL.

        ii. Add 1 mL of the laminin coating solution to each well of a 6-well plate (or adjust the volume for alternative plate formats), ensuring that the entire surface is evenly covered.

        iii. Incubate for 2 hours at 37°C, or overnight at 2°C to 8°C. If incubating overnight, consider sealing the plate with Parafilm before placing it in the incubator.

3. Cell density: Seed 135,000 cells per well in a 6-well plate or 54,000 cells per well in a 12-well plate. Adjust the cell density accordingly when scaling up or down based on the vessel surface area. Ensure thorough mixing for even distribution to prevent the formation of large colonies.

Day 2

- Remove Day 0 Medium and exchange for Day 2 Medium.

Day 4

- Remove Day 2 Medium and replace it with Day 4 Maturation Medium.

Day 6

- Remove Day 4 Medium and replace it with Day 4 Maturation Medium.

From Day 8 until the end of the culture period

- Remove the Day 4 medium and replace it with the Day 8 Maturation Medium, which contains CX3CL1 and excludes doxycycline.

Media Compositions

Day 0 Medium

Component	Stock Conc.	Final Conc.	Dilution	Vendor, cat. #
mTeSR™ Plus Medium				STEMCELL Technologies, #100-0276
Doxycycline	2 mg/mL	2 µg/mL	1:1000	Sigma-Aldrich, #D9891-1G
RevitaCell™ (100x)	100x	1x	1:100	Thermo Fisher Scientific, #A2644501

Day 2 Medium

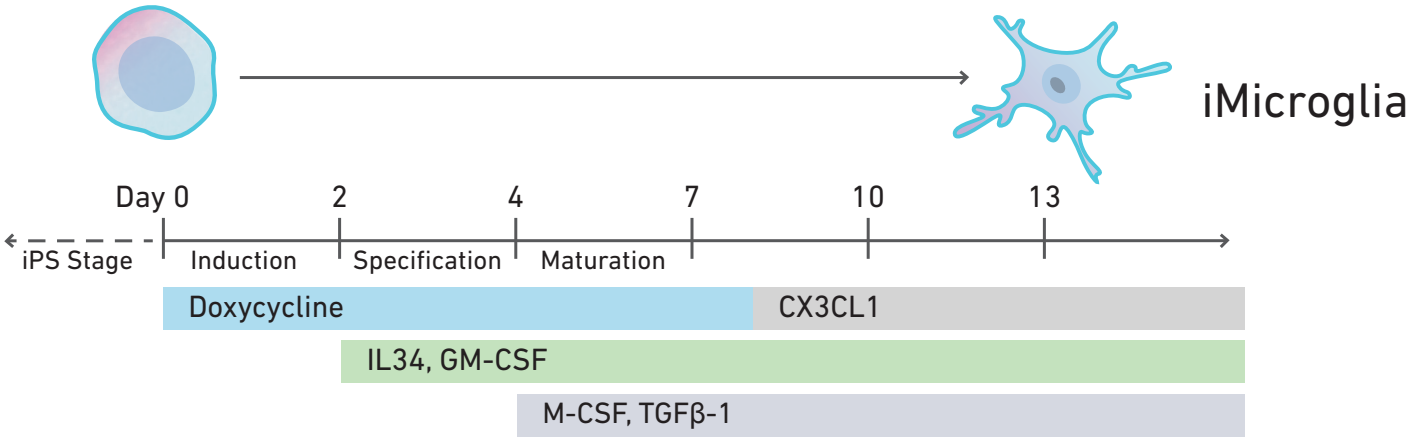
Component	Stock Conc.	Final Conc.	Dilution	Vendor, cat. #
Advanced DMEM/F-12				Thermo Fisher Scientific, #12634010
GlutaMAX™	100x	1x	1:100	Thermo Fisher Scientific, #35050061
Doxycycline	2 mg/mL	2 µg/mL	1:1000	Sigma-Aldrich, #D9891-1G
Human IL-34	100 µg/mL	100 ng/mL	1:1000	BioLegend, #577906
Human GM-CSF	50 µg/mL	10 ng/mL	1:5000	BioLegend, #572904

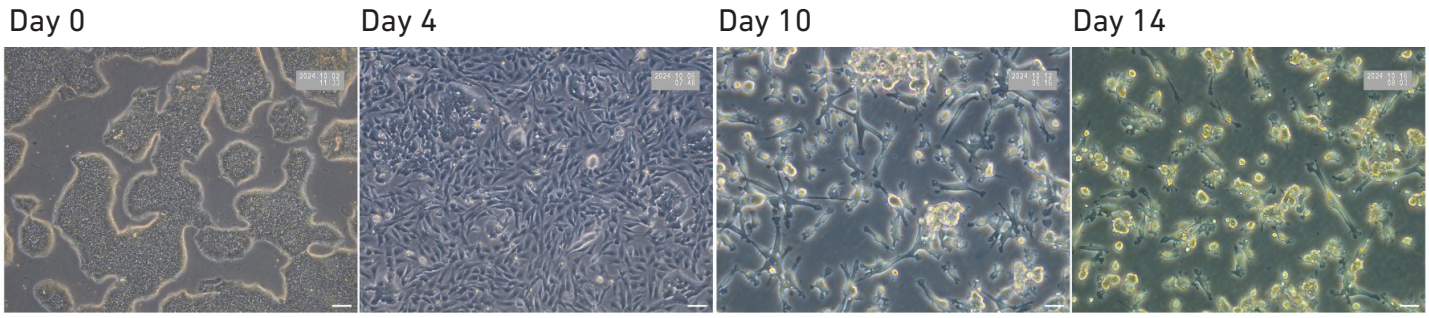
Day 4-6 Medium

Component	Stock Conc.	Final Conc.	Dilution	Vendor, cat. #
Advanced DMEM/F-12				Thermo Fisher Scientific, #12634010
GlutaMAX™	100x	1x	1:100	Thermo Fisher Scientific, #35050061
Doxycycline	2 mg/mL	2 µg/mL	1:1000	Sigma-Aldrich, #D9891-1G
Human IL-34	100 µg/mL	100 ng/mL	1:1000	BioLegend, #577906
Human GM-CSF	50 µg/mL	10 ng/mL	1:5000	BioLegend, #572904
Human M-CSF	100 µg/mL	50 ng/mL	1:2000	BioLegend, #574806
Human TGF-β1	50 µg/mL	50 ng/mL	1:1000	Peprotech, #100-21C

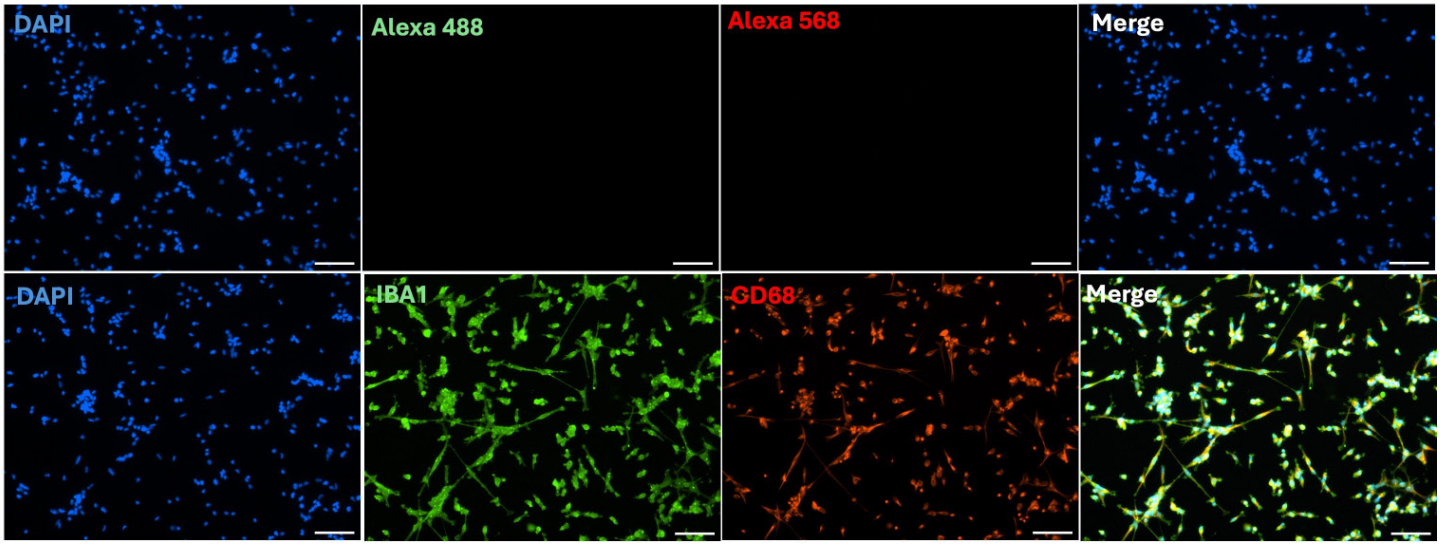
Day 8-15 Medium

Component	Stock Conc.	Final Conc.	Dilution	Vendor, cat. #
Advanced DMEM/F-12				Thermo Fisher Scientific, #12634010
GlutaMAX™	100x	1x	1:100	Thermo Fisher Scientific, #35050061
Human IL-34	100 µg/mL	100 ng/mL	1:1000	BioLegend, #577906
Human GM-CSF	50 µg/mL	10 ng/mL	1:5000	BioLegend, #572904
Human M-CSF	100 µg/mL	50 ng/mL	1:2000	BioLegend, #574806
Human TGF-β1	50 µg/mL	50 ng/mL	1:1000	Peprotech, #100-21C
Human CX3CL1	100 µg/mL	50 ng/ml	1:1000	Peprotech # 30031





Longitudinal phase-contrast images of KOLF2.1J CLYBL-6TF-iMG cells following doxycycline treatment.
On day 0 (prior to doxycycline treatment), iPS cells maintain a pluripotent state. Upon doxycycline addition, iPS cells begin differentiating into microglia.



KOLF2.1J CLYBL-6TF-iMG, 15 days post-doxycycline treatment.
Representative immunofluorescence images on day 15 of differentiation. Cells were stained for microglial markers IBA1 and CD68, with nuclei counterstained using DAPI. Negative controls were processed with secondary antibody only.

The differentiation protocol and images were kindly provided by Nélio Oliveira (The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA).