THP-G8 cell Culture Protocol

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This protocol describes how to culture THP-G8 cells, THP-1-derived IL-8 reporter cell line, for the IL-8 Luc assay.

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【1】Reagents

- RPMI-1640 (GIBCO Cat#11875-093, 500 mL)
- FBS (SIGMA #172012-500ML)
- 100 x concentrated antibiotic and antimycotic (10000 U/mL of Penicillin G, 10000 μg/mL of Streptomycin and 25 μg/mL of Amphotericin B in 0.85 % Saline) (e.g., GIBCO Cat#15240-062)
- G418 (CAS:108321-42-2, Nacalai Tesque Cat#09380-86)
- Puromycin (CAS:58-58-2, InvivoGen Cat#ant-pr-1)
- T-75 flask tissue culture treated (e.g., Corning Cat#353136)
- CELLBANKER 1 (Zenoaq Resource Co., Ltd Cat# CB011, 100 mL)

【2】Medium

A medium：for maintenance of THP-G8 cells

Reagent	Company	Concentration	Final concentration in medium	Required amount
RPMI-1640	GIBCO #11875-093	-	-	445 mL
FBS	SIGMA #172012-500ML	-	10 %	50 mL
Antibiotic-Antimycotic	GIBCO #15240-062	100×	1×	5 mL
Puromycin	InvivoGen # ant-pr-1	10 mg/mL	0.15 μg/mL	7.5 μL
G418	Nacalai tesque #09380-86	50 mg/mL	300 μg/mL	3 mL

B medium：for luciferase assay

Reagent	Company	Concentration	Final concentration in medium	Required amount
RPMI-1640	GIBCO #11875-093	-	-	27 mL
FBS	SIGMA #172012-500ML	-	10 %	3 mL

C medium：for thawing THP-G8 cells

Reagent	Company	Concentration	Final concentration in medium	Required amount
RPMI-1640	GIBCO #11875-093	-	-	26.7 mL
FBS	SIGMA #172012-500ML	-	10 %	3 mL
Antibiotic-Antimycotic	GIBCO #15240-062	100×	1×	0.3 mL

【3】Culture

Cryopreservation → Culture

- ☐ Prepare 30 mL of C medium in a 50 mL tube and warm it in a 37°C water bath.
- ☐ Dispense 9 ml of C medium into a 15 mL tube and 14 ml into a T-75 flask.
- ☐ THP-G8 Stock is half-thawed in a 37°C water bath and rapidly dissolved by adding 1 mL from 9 ml dispensed into a 15 ml tube with the ice block remaining, and collected in a 15 ml tube.
- ☐ Centrifuge at 1,300 rpm for 5 minutes at room temperature.
- ☐ Remove the supernatant, resuspend in 1 mL of C medium, and count the cells.
- ☐ Add 1 mL of the resuspended cell solution to 14 mL of C medium in a T-75 flask and seed the cells in to the T-75 Flask.
- ☐ Incubate for 4 days at 37ºC, 5% CO_2.

Medium change (C medium → A medium)

- ☐ Prepare 20 mL of A medium in a 50 mL tube and warm it in a 37°C water bath.
- ☐ Count the cells in culture with the T-75 Flask.
- ☐ Harvest the cells from the T-75 Flask into a 15 mL tube.

- ☐ Centrifuge at 1,300 rpm for 5 minutes at room temperature.
- ☐ Remove the supernatant, resuspend in 4 mL of warmed A medium, and count the cells.
- ☐ Seed cells at about 5.0 x 10^5 cells/mL in a T-75 Flask (15mL/flask).
- ☐ Incubate at 37ºC, 5% CO2 for 3-4 days (check the cell condition and incubate until 1.5-2.0 x 10^6 cells/mL).

Passage

- ☐ Count the cells in culture with the T-75 Flask.
  *Check that the cells growth to 1.5×10^6 cells/mL and passaging. Do not allow the cell number to exceed 2.0 x 10^6 cells/mL.
- ☐ Prepare A medium and warm it in a 37°C water bath.
- ☐ Harvest the cells from the T-75 Flask into a 15 mL tube.
- ☐ Centrifuge at 1,300 rpm for 5 minutes at room temperature.
- ☐ Remove the supernatant, resuspend in 4 mL of warmed A medium, and count the cells.
- ☐ Seed cells at about 5.0 x 10^5 cells/mL in a T-75 Flask (15mL/flask).
- ☐ Incubate at 37ºC, 5% CO2 for 3-4 days (check the cell condition and incubate until 1.5-2.0 x 10^6 cells/mL).

Cryopreservation

- ☐ Count the cells in culture with the T-75 Flask.
  *Check that the cells growth to 1.5×10^6 cells/mL and passaging. Do not allow the cell number to exceed 2.0 x 10^6 cells/mL.
- ☐ Prepare CELLBANKER1 on ice.
- ☐ Harvest the cells from the T-75 Flask into a 15 mL tube (or 50 mL tube).
- ☐ Centrifuge at 1,300 rpm for 5 minutes at room temperature.
- ☐ Remove the supernatant and tapping the tube to loosen the pellet.
- ☐ Suspend the cells to 2×10^6 cells/500 µL in CELLBANKER 1, dispense 500 µL into each cryotube, and store immediately at -80°C.
- ☐ The next day, transfer the stock at -80°C to the gas phase of liquid nitrogen for storage.