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ESGRO-2i MEDIUM

CATALOG NUMBER: SF016-100 **QUANTITY:** 100 mL

LOT NUMBER:

DESCRIPTION: ESGRO-2i Medium is a defined, LIF containing medium provided with a selective GSK3B inhibitor and Mek1/2 inhibitor for the culture of mouse embryonic stem (ES) and induced pluripotent stem (iPS) cells and increased maintenance of pluripotency. This medium is intended for mouse pluripotent stem cell expansion and growth at clonal density in serum-free conditions.

APPLICATIONS:

- Mouse ES cell derivation, passaging and maintenance
- Mouse iPSC generation, expansion and maintenance
- Media can be used on feeder dependent/independent ES cell lines

COMPONENTS:

1. ESGRO-2i Basal Medium with LIF: (Catalogue No. CS204463) One (1) 100 mL bottle containing basal medium supplemented with LIF, pH 7.1-7.3.
2. GSK3β Inhibitor Supplement: (Catalogue No. SF012-050) One (1) 50 µL vial containing selective GSK3β inhibitor supplement.
3. Mek1/2 Inhibitor Supplement: (Catalogue No. CS204465) One (1) 10 µL vial containing selective Mek 1/2 inhibitor supplement.

MEDIA PREPARATION:

- Thaw basal medium at 37°C.
- Thaw inhibitor supplements at room temperature.
- Aseptically transfer the contents of the vials containing the supplements to the pre-warmed bottle of basal medium and mix.

STORAGE AND HANDLING:

- Upon receipt, store basal medium and inhibitor supplements in the dark at -20°C until use. Refer to lot expiration date on labels.
- After basal medium and inhibitor supplements are combined, store the complete ESGRO-2i medium in the dark at 4°C for up to 10 days. Do not refreeze.

MATERIALS REQUIRED BUT NOT PROVIDED:

ESGRO Complete Basal Medium (Cat. No. SCR002-500)

ESGRO Complete Gelatin (Cat. No. SF008)

ESGRO Complete Accutase™ (Cat. No. SF006)

D-PBS (part no. BSS-1006-B)

ADAPTATION PROTOCOL (STEPS 1-13):

The following protocol is for the direct adaptation of both feeder-dependent and feeder-independent mouse ES (mES) cells from serum-containing medium to serum-free cell culture conditions in ESGRO-2i medium. For regular culture in ESGRO-2i medium, follow steps 7-13 below.

- Pre-warm all reagents to 37°C prior to use.
- Avoid using glassware, as ES cells in serum-free media are sensitive to any residual detergent. The use of disposable plastic ware in any manipulations is strongly recommended.

If ES cells were cultured in serum-containing medium, the first split can be done in your regular trypsin, and then all subsequent passaging is performed in Accutase. Feeder cells naturally deplete within 3-4 passages, at which time the cells are considered adapted.

1. Grow mES cells to 60% confluence in serum-supplemented medium in a 6-well plate with or without feeders.
2. Pre-coat T25 flasks or 6-well plates with Gelatin Solution (part no. SF008).
3. Wash cells once with D-PBS (part no. BSS-1006-B). To dissociate cells, add 0.05% trypsin and incubate at 37°C for 5-10 minutes.
4. Pipet up and down to ensure a single cell suspension and transfer cells to a 15 mL tube with 10 mL of pre-warmed serum-containing medium.
5. Spin cells down at 1000rpm, discard supernatant, and resuspend in ESGRO-2i medium.
6. Count cells and plate at a density of: 0.5-5 x10^5 cells per 6-well in a volume of 4 mL ESGRO-2i medium. ***NOTE:*** Depending on the density of the cell culture, it may be necessary to passage the cells the next day.

iPSC AND MOUSE ESC MAINTENANCE IN ESGRO-2i MEDIUM (STEPS 7-12):

7. Completely remove medium (no need to wash cells with PBS). To dissociate cells, add 0.5-1 mL Accutase (part no. SF006) per 6-well. Incubate at RT and allow cells to detach (2-5 minutes). Do not let cells become confluent.

   ***IMPORTANT NOTE*:** Do not use standard trypsin from this point onward. Standard trypsin affects the attachment properties of the cells, which form clusters of free floating cells.
8. Pipette up and down to obtain a single cell solution. To wash the cells, prepare a 15 mL tube with 5 mL of Basal Medium (part no. SF002-500), or DMEM/F12, and add the single cell suspension, mix and spin at 1000 rpm.
9. Repeat the wash: Remove supernatant, add again 5 mL of Basal Medium or DMEM/F12, and resuspend cells.
10. Count the cells and spin at 1000rpm. Remove supernatant and resuspend pellet in ESGRO-2i medium.
11. Passage cells at a ratio of 1:2 – 1:5 and add in 4 mL pre-warmed ESGRO-2i Medium per 6-well.

12. Observe cell growth over the next 1-3 days. Some residual feeders remain, and initially some cell death may be observed. However, mES cell colonies will continue to grow and will appear bright with a similar morphology as in serum-containing medium. When mES cells are 60-90% confluent, cells may be passaged again.
13. Repeat step 7-11 once or twice more for a total of 1-2 passages, until all feeder cells are depleted. Depending on the growth vigour of the cells, a 1:5 split routine can be adopted every 2-3 days.

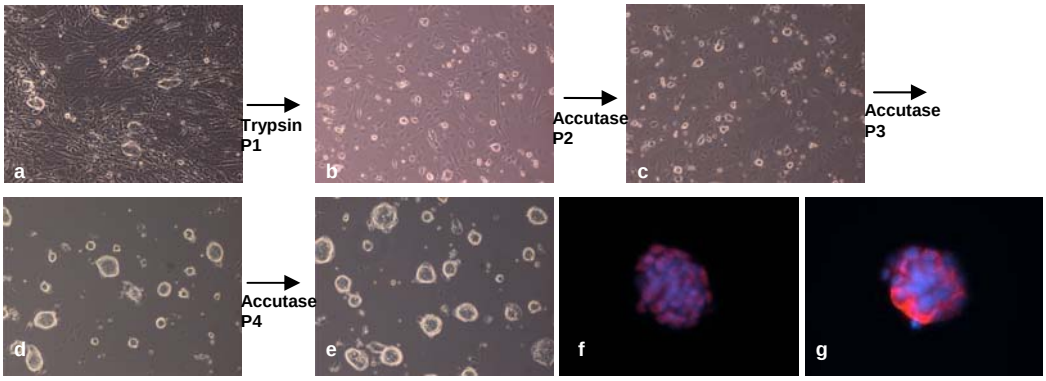

Figure 1. Adaptation time course with CMTI-1 cells. On day 1 (a) the first passage (P1) is done with trypsin with regular feeder dependent mESCs (CMTI-1). Subsequent passages are done with Accutase. In this adaptation cells were passaged on day 2 (b) and day 3 (c). From day 5 (d) onwards no feeder cells remain. At day 7 after P4 (e), ESGRO-2i adapted ES colonies were stained with anti-Oct4 (f) and anti-SSEA-1(g) antibodies, both red and overlaid with blue DAPI nuclear staining.

Figure 2. Generation of chimera from injection of 129 ES cells cultured in ESGRO-2i medium into C57BL/6 host blastocysts. Agouti patches on coat signify contribution of injected ES cells.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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