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# ES2N COMPLETE MEDIUM KIT

**CATALOG NUMBER:** SCM082 **QUANTITY:** 1 Kit (250 mL)

**LOT NUMBER:**

**DESCRIPTION:** ES2N Complete medium is a defined serum-free formulation that has been optimized for the differentiation of mouse embryonic stem (ES) and induced pluripotent stem (iPS) cells into neurons. When used in conjucture with ESGRO Complete Plus medium mouse ES and iPS cells readily differentiate into neurons in a monolayer assay within 9-12 days on gelatin coated culture dishes.

**APPLICATIONS:**

- Neuronal monolayer differentiation of mouse ES and iPS cells in serum-free conditions.
- ES2N Complete medium supplemented with EGF and FGF enables the maintenance and expansion of neural stem cells (NSCs).

**QC PROTOCOL:** Each lot of ES2N Complete Medium is tested in a 9-12 day neuronal differentiation assay.

**COMPONENTS:**

- ES2N Basal Medium: (Cat. No. SCM083) One (1) 245 mL bottle containing standard base medium.
- Neuro27 Medium Supplement: (Cat. No. SCM013-S) One (1) 5 mL vial containing neural supplement (use as 50x).
- Neuro2 Medium Supplement: (Cat. No. SCM012-S) One (1) 1.25 mL vial containing N2-like supplement (use as 200x).

**MEDIA PREPARATION:** Pre-warm ES2N Basal medium at 37°C and thaw the Neuro27 and Neuro2 supplements at room temperature (RT). Transfer the contents of the vials containing the supplements to the thawed bottle of base medium and mix to constitute the complete medium.

**STORAGE/HANDLING:**

- Upon receipt, store ES2N Basal medium and supplements in the dark at -20°C. Refer to lot expiration date on labels.
- After supplements are thawed at room temperature, combine with base medium and store in the dark at 2-8°C for up to 2 weeks or at -20°C for up to 2 months. Avoid repeat freeze/thaw cycles.

**MATERIALS REQUIRED BUT NOT PROVIDED:**

ESGRO Complete Plus Clonal Medium (Cat. No. SF001-500P; SF001-100P)
ESGRO Complete Basal Medium (Cat. No. SF002-500; SF002-100)
ESGRO Complete Gelatin (Cat. No. SF008)
Accutase™ Solution (Cat. No. SF006)
D-PBS (Cat. No. BSS-1006-B)
Basic FGF; FGF-2 (Cat. No. GF003)
EGF (Cat. No. GF144)

**PREPARATION OF MOUSE ES & IPS CELLS:**

The following protocol is for the direct differentiation into neurons from both feeder-dependent and feeder-independent mouse ES and iPS cells.

Serum-free and feeder-free adapted mouse ES and iPS cells

1. 3-4 days prior to the experiment, thaw and culture in ESGRO Complete Plus medium.
2. Passage cells by adding the least amount of Accutase to cover the cells, gently tap the culture dish.
3. Transfer the single cell solution into a vial with 5-10 mL ESGRO Complete Basal medium. Spin down for 5 minutes at 1000 rpm and remove supernatant. At this point a very small amount of cells (10 µL) can be removed for a cell count.
4. Repeat the wash in step 3.
5. Plate cells in an appropriate sized culture vessel coated with 0.1% Gelatin. As a rule it is best to passage cells once or twice in the ESGRO Complete Plus medium starting with the neuronal differentiation assay.
6. Start with step 1 of the Neuronal Differentiation Assay with a confluent ES cell culture.

Serum and feeder dependent mouse ES and iPS cells

1. Wash cells with PBS.
2. Add the least amount of trypsin to cover the cells, tap the culture dish, and transfer the single cell suspension in a vial with 5-10 mL serum-containing medium.
3. Spin at 1000 rpm for 5 minutes, discard supernatant.
4. Repeat wash using 5-10 mL ESGRO Complete Basal Medium. Remove a small amount of resuspended cells for a cell count and spin 5 minutes at 1000 rpm. Discard supernatant.
5. Resuspend cells in ESGRO Complete Plus medium and plate cells on 0.1% Gelatin coated dishes.
6. Culture at 37°C with 5% $CO_2$ until confluent. Passage cells until feeder cells are depleted. Generally, cells are feeder free within 2-3 passages.

**NEURONAL DIFFERENTATION ASSAY:**

On the day of the neuronal differentiation experiment:

1. Coat 6-well dishes with 0.1% gelatin 1- 4 hours at room temperature. *Note: Use fresh Gelatin. Do not coat for less than one hour or more than 4-6 hours.*
2. Passage serum-free and feeder-free mouse ES and iPS cells with Accutase, wash twice and count cells with a hemocytometer.
3. Plate 1-3 x $10^4$ cells/$cm^2$ (1-3x $10^5$ cells per 6-well plate). *Note: Plating the correct density is crucial. Each cell line needs optimization.*
4. Remove gelatin and add correct number of cells in 30 – 200 µL volume of ES2N Complete medium.
5. Add 3 - 4 mL of ES2N Complete medium to each well and place at 37°C incubator with 5% $CO_2$.
6. Change medium every 1-2 days, gently aspirate undifferentiated cells. Depending on the cell line there can be cell death around day 3-5 associated with the assay.

The assay is completed on day 9-12, with up to 80-90% of neurons generated. Neurons may be kept in medium for an additional 4-7 days for analysis. During the assay, neural stem cells (NSCs) and precursor cells form rosettes on day 4-6. At this point NSCs can be isolated by replating cells in ES2N Complete mediuim containing bFGF and EGF and following the protocol for neural stem cell maintenance below.

**NEURAL STEM CELL MAINTENANCE/ EXPANSION:**

Protocol for isolation and maintenance of Neural Stem Cells (NSCs):

1. Aseptically add bFGF and EGF (both at 20ug/mL final concentration) to the ES2N Complete medium.
2. Coat 6-well dishes with matrix, if required. *Note: the strongest adherence of NSCs occurs on fibronectin coated wells, followed by laminin/polyornithine, and lastly gelatin.*
3. Briefly wash cells with PBS. Add just enough Accutase to cover adherent cells and incubate at RT for 2-4 minutes.
4. Tap on the culture vessel and pipet up and down to obtain a single cell suspension. Collect cells in Accutase and transfer into a vial containing 5mL prewarmed ES2N Basal Medium.
5. Centrifuge for 5 minutes at 1000 rpm and resuspend in ES2N Basal Medium. This is your first wash.
6. Count cells with the hemocytometer. Spin cells down for 5 minutes at 1000rpm, resuspend in ES2N Basal Medium. Spin down again for 5 minutes at 1000rpm, this is your second wash.
7. Discard supernatant and resuspend cells in ES2N complete Medium containing bFGF and EGF, and transfer to a new culture well.
8. Plate 1-3 x $10^4$ cells/$cm^2$ (1-3x $10^5$ cells/6-well).
9. Add medium to a final 3 - 4 ml of ES2N complete medium containing EGF and bFGF to each 6-well and place at 37°C incubator with 5% $CO_2$.
10. Change medium every day.

## CELL MORPHOLOGY*:

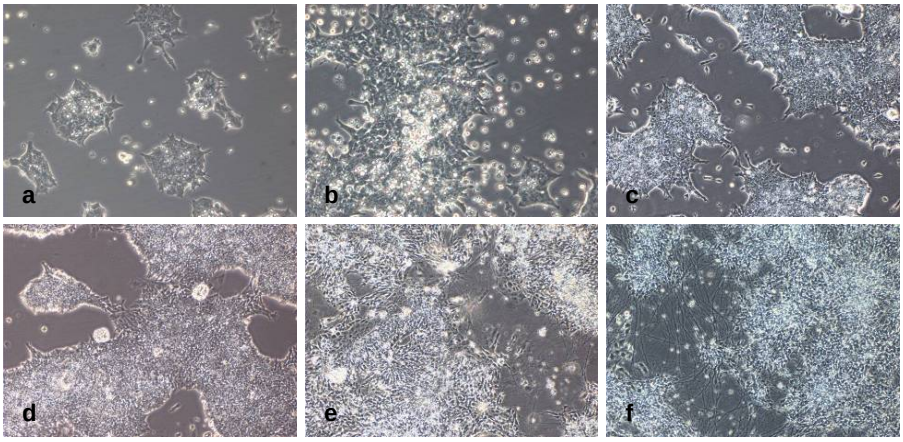
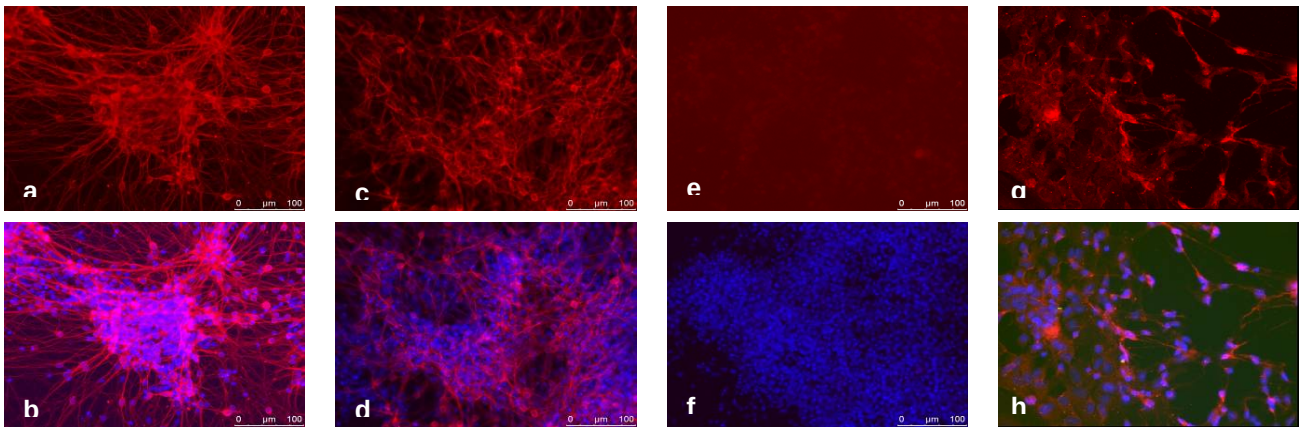



**Fig 1. Morphology and time course of neuronal differentiation from CMTI-1 (129SvEv) mES cells.** **a)** Day1, small flat colonies appear. **b)** Day 2, note that undifferentiated cells start to float. **c)** Day 3, continued floating of undifferentiated and dead cells. **d)** Day 4, continued floating of undifferentiated and dead cells. **e)** Day 7, neuronal rosettes have formed. **f)** Day 9, Differentiated neurons can be seen at the earliest at day 9, by day 12 most cells have are differentiated.

## CELL ANALYSIS*:




* Images based upon representative data from a previous lot.

**Fig 2. Staining of differentiated neurons from SCR012 (129S6) mES cells at day 12.** **a)** Tubulin red staining (Cat. No. MAB1637). **b)** Overlay of Tubulin and nuclear DAPI staining, note that more than 80% of cells stain Tubulin positive. **c)** MAP-2 red staining (Cat. No. MAB4318). **d)** Overlay of MAP-2 and DAPI staining. **e)** Oct-4 staining (Cat. No. MAB4419), note absence of Oct-4 staining, indicating that most of the stem cells have differentiated. **f)** Overlay of Oct-4 and DAPI showing only nuclear DAPI staining. **g)** GFAP (red) staining (Cat. No. AB5804). **h)** Overlay of GFAP/DAPI staining.

***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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