



Rescue of ES Cell Lines using RESGRO Culture Medium

RESGRO Culture Medium has the capacity to rescue traditional ES cell lines that have started drifting and either generate low percentage chimeras or have lost germline transmission capability. Differentiated ES cells not visible with traditional ES cell culture medium, will become visible with RESGRO medium. After 2 passages, a clear difference is seen between differentiated and undifferentiated ES cells. At that moment, it is recommended to perform a subcloning to select the undifferentiated cells.

The selection procedure should be repeated if some differentiation is still present after one subcloning procedure.

Materials & Reagents required:

- RESGRO Culture Medium (Cat. No. SCM001 or SCM002)
- EmbryoMax ES Cell Qualified L-Glutamine Solution (Cat. No. TMS-002-C)
- Stericup-GP Filter Unit, 0.22 µm, PES, 500 mL (Cat. No. SCGPU05RE)
- Centrifuge
- ES Cell Medium:
 - DMEM (Cat. No. SLM-220-B)
 - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
 - 1% Nucleosides, 100x (Cat. No. ES-008-D)
 - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
 - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
 - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
 - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
 - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- Incubator, 37 °C/5% CO₂
- Pipette
- PMEF Feeder cell coated culture plates
- 0.05% Trypsin-0.53mM EDTA (Cat. No. SM-2002-C)
- Water Bath, 37°C

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Procedure:

1. Prepare RESGRO Culture Medium for use by adding 10 mL of ES Cell Qualified L-Glutamine Solution (200mM) to 500 mL of RESGRO Culture Medium. If required, filter the solution using only cellulose acetate, PVDF or PES filters.
2. Culture the ES cells in RESGRO Culture Medium for 2 passages on a monolayer of PMEF feeder cells.
3. After 2 passages, replate 1/3–1/5 of the cell suspension on the same size plate without PMEF feeder cells.
4. After 2 days, a clear difference will be observed between 3-dimensional (undifferentiated) and flat growing (differentiated) colonies. By tapping the dish, the undifferentiated colonies will detach.
5. Collect the supernatant (which will contain the undifferentiated cells) and discard the dish containing the differentiated cells.
6. Centrifuge the supernatant and remove the medium.
7. Add 0.5 mL of Trypsin-EDTA to the cell pellet.
8. Pipette up and down with a 1 mL pipette (do not use pipette tip of smaller volume).
9. Place the cell suspension in a water bath at 37 °C for 1.5 minutes.
10. Pipette up and down, 10 times (with a 200 µL pipette tip or a 1 mL pipette).
11. Add 9.5 mL of RESGRO Culture Medium.
12. Centrifuge and remove the supernatant.
13. Add an appropriate volume of RESGRO Culture Medium, which will depend upon the final volume that you prefer to plate the cells. For 6-well plates, it is recommended that the cells be suspended in 4 mL of RESGRO Culture Medium. Plate 1/3–1/6 of the ES cells on wells containing mitotically inactivated PMEF feeder cells. Alternatively, ES cells can be cultured in ES Cell Medium containing ESGRO supplement.

Note: Avoid contact between the colonies. If the ES cells have been plated at too high a density, replate ES cells at a lower density the following day.

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Table 14.1:
Improved efficiency of Murine ES cell lines using RESGRO Culture Medium

ES Cell Line	Medium* & Method used	Number of Embryos Transferred	Number of Pups Born	Number of Chimeras Born	Percentage Chimerism
R1#19 Knockout clone	Traditional medium Blastocyte injection	56	7	1	1 x 10%
R1#19 Knockout clone	RESGRO medium Blastocyte injection	64	27	20	3 x 5%
					3 x 10%
					1 x 20%
					2 x 30%
					4 x 40%
					2 x 50%
					2 x 60%
					2 x 70%
1 x 80%					
129SvEv Wildtype clone	Traditional medium Diploid aggregation	40	28	4	1 x 2%
					1 x 5%
					1 x 10%
					1 x 50%
129SvEv Wildtype clone	RESGRO medium Diploid aggregation	106	25	25	11 died
					1 x 10%
					1 x 90%
					12 x 100%
C57B1/6 Knockout clone	Traditional medium Blastocyte injection	50	8	0	0
C57B1/6 Knockout clone	RESGRO medium Blastocyte injection	96	38	19	2 died
					1 x 2%
					3 x 5%
					4 x 10%
					1 x 20%
					2 x 30%
					1 x 60%
					3 x 70%
2 x 80%					

*Traditional medium: basal medium supplemented with ESGRO