



ES Cell Line Derivation using RESGRO Culture Medium

The efficiency of ES cell derivation is greatly strain dependent. To date, very few murine ES cell lines are available from inbred strains other than 129 strains, and those derived have generally been obtained with low success rates. Furthermore, ES cells derived from strains other than 129 are in general more difficult to propagate *in vitro*. Especially at high passage number and after genetic manipulation, these cell lines generate chimeras less efficiently and contribute less frequently to the germline.

RESGRO Culture Medium enables the efficient derivation and maintenance of ES cell lines from several inbred mouse strains, including certain strains that were previously considered to be non-permissive for ES cell derivation. A recent study demonstrated that RESGRO medium allowed the derivation of ES cell lines from inbred strains other than 129 (including FVB, a strain previously considered to be non-permissive for ES cell derivation and C57Bl/6N, BALB/c, 129/SvEv and DBA/2N mouse strains).

The following protocol is based upon that used by Schoonjans L. *et al.* (Stem Cells 21:90-97. 2003). Please refer to this reference for comprehensive details on the application of RESGRO Culture Medium for ES cell derivation.

Table 15.1:
Efficiency of ES Cell Derivation and Germline Competence with RESGRO Culture Medium

Mouse Strain	Blastocysts Cultured (n)	Established ES Cell Lines (n)	Established ES Cell Lines (%)	No. Germline Competent ES Cell Lines/ No. ES Cell Lines Cultured
C57Bl/6N	35	18	51	10/11
FVB/N	20	8	40	6/9
BALB/c	34	15	44	7/7
129SvEv	10	6	60	4/4
DBA-2/N	34	13	38	3/3

ES Cell Line Derivation using RESGRO Culture Medium

Materials & Reagents required:

- RESGRO Culture Medium (Cat. No. SCM001, Cat. No. SCM002)
- EmbryoMax ES Cell Qualified L-Glutamine Solution (Cat. No. TMS-002-C)
- 96-well plates coated with PMEF Feeder cells
- Stericup-GP Filter Unit, 0.22 µm, PES, 500 mL (Cat. No. SCGPU05RE)
- Incubator, 37 °C/5% CO₂
- 0.25% Trypsin-1mM EDTA (Cat. No. SM-2003-C)

Procedure:

1. Collect 3.5 to 4.5 day old blastocyst stage mouse embryos and plate on a 96-well dish covered with a freshly prepared monolayer of PMEF feeder cells.
2. Prepare RESGRO Culture Medium for use by adding 10 mL of ES Cell Qualified L-Glutamine Solution (200 mM) to 500 mL of RESGRO Culture Medium. If required, filter the solution using only cellulose acetate, PVDF or PES filters.
3. During the first 2 days remove only 75% of the medium and replace gently with fresh RESGRO Culture Medium (in order to avoid detachment of the blastocysts).
4. After attachment of the blastocysts, replace the medium completely on a daily basis with RESGRO Culture Medium.
5. After 5–6 days in culture, remove the inner cell mass (ICM) outgrowth from the trophoectoderm. Replate the cells following trypsinization with 0.25% Trypsin-1mM EDTA on a 96-well plate covered with a monolayer of PMEF feeder cells.
6. Culture the ES cells until 70–80% confluent, and then replate on larger culture dishes.
7. Passage ES cells every 2–4 days on freshly prepared feeder layers, and replace with fresh RESGRO Culture Medium daily.