



Rescue, Derive and Expand with RESGRO™ Culture Medium

Novel Mouse ES Cell Culture Medium

Abstract

RESGRO Culture Medium is a complete ready-to-use cell culture medium that complements traditional mouse Embryonic Stem (ES) cell culture media containing ESGRO® mouse LIF medium supplement.

In contrast to traditional medium, RESGRO Culture Medium is recommended for a number of specialized applications including the rescue of established ES cell lines and the derivation of new ES cell lines.

Rescue of Established ES Cell Lines

RESGRO Culture Medium has the capacity to rescue established ES cell lines that have started drifting and either generate low percentage chimeras or have lost germline transmission capability. Differentiation, when present in ES cell culture but not visible with traditional medium, will become recognizable when using RESGRO Culture Medium. After 2 passages, a clear difference is seen between differentiated and undifferentiated ES cells, at

which time undifferentiated cells can be removed by sub-cloning. The application of RESGRO medium to improve the efficiency of mouse ES cell lines that had generated a low percentage of chimeras and germline transmission

Table 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 Blastocyst injection	56	7	1	1 x 10 %
R1#19 Knockout clone	RESGRO medium Blastocyst 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 Wild-type clone	Traditional medium Diploid aggregation	40	28	4	1 x 2 % 1 x 5 % 1 x 10 % 1 x 50 %
129SvEv Wild-type clone	RESGRO medium Diploid aggregation	106	25	25	11 died 1 x 10 % 1 x 90 % 12 x 100 %
C57Bl/6 Knockout clone	Traditional medium Blastocyst injection	50	8	0	0
C57Bl/6 Knockout clone	RESGRO medium Blastocyst 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® mLIF Medium Supplement.

capability was demonstrated (Table 1). In all cases, the subculturing the cell lines with RESGRO medium resulted in an improved proportion of chimeras born and an increased percentage of chimeric progeny.

Derivation of Mouse ES Cells

Genetically altered mice derived by homologous recombination in 129 ES cell lines may exhibit highly variable phenotypes due to variation in genetic background, indicating that genes unrelated to the targeted genes can markedly affect the observed phenotype. Backcross breeding diminishes overall genetic heterogeneity, but selection for the targeted locus maintains flanking parental genomic DNA, precluding generation of identical congenic experimental and control mice. Elimination of genetic background variability requires derivation of germline competent ES cell lines from inbred mouse strains with specific genetic backgrounds, enabling generation of isogenic gene-targeted and control mice.

The efficiency of ES cell derivation is greatly strain dependent. To date, very few mouse 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 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

Table 2. 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 cellines/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

(Figures 1 and 2). A recent study demonstrated that RESGRO medium allowed the derivation of ES cell lines from 5 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 strains¹.

ES cell lines were derived from all of 5 inbred mouse strains tested and the efficiency of ES cell line derivation ranged from 38% – 60% (Table 2). Furthermore, all ES cell lines tested resulted in chimeric offspring, as judged from the contribution to the coat color of the strain from which the ES cell lines were derived (Figure 3). These chimeras had the ability to pass the ES cell genome to the next generation, as judged from offspring with the coat color of the ES cell strain after mating with relevant recipient females.

References

1. Schoonjans L et al. (2003). *Stem Cells* **21**(1):90-7.

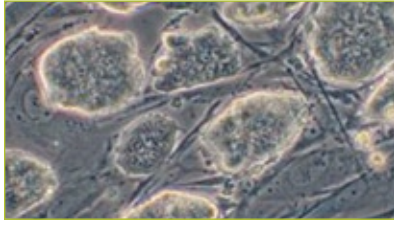


Figure 1. DBA-2N embryonic stem cells passage 67 on mouse embryonic fibroblast cells.

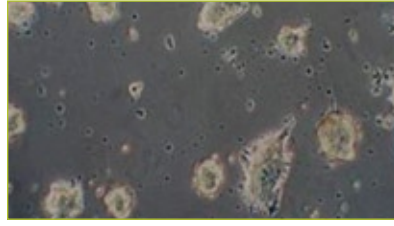


Figure 2. C57Bl/6N ES cells on bare dish



Figure 3. Offspring born after injection of FVB/N #17 passage 18 ES cells into C57Bl/6N blastocysts. Left mouse shows 100 % chimerism, right mouse shows 5 %.

Description	Quantity*	Cat. No.
RESGRO Culture Medium	250 mL	SCM001
RESGRO Culture Medium	500 mL	SCM002



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