EMBRYONIC **STEM CELL CULTURE MEDIUM**

RESGRO™ Culture Medium

CHEMICON® INTERNATIONAL
a Serologicals® Company

Rescue, Derive & Expand with RESGRO™

Novel ES Cell Culture Medium

Chemicon International is proud to introduce RESGRO™ Culture medium, a complete ready-to-use cell culture medium that can be utilized to complement traditional murine Embryonic Stem (ES) cell culture media containing ESGRO® (murine LIF). In contrast to traditional medium, RESGRO™ Culture Medium is recommended for a number of specialized applications including:

- The derivation of new ES cell lines
- Rescue of established ES cell lines
- Stroma free expansion of $CD34^+$ stem cells

In conjunction with our existing products for stem cell research including ESGRO®, Leukemia Inhibitory Factor, antibodies and kits, Chemicon provides researchers with innovative products to facilitate stem cell research.

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, which is present in ES cells 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™ to improve the efficiency of a number of murine ES cell lines (R1, 129SvEV and C57Bl/6) that had generated a low percentage of chimeras and germline transmission capability was demonstrated (Table 1). In all cases, the sub-culture of these cell lines with RESGRO™ resulted in an improved proportion of chimeras born and an increased percentage of chimeric progeny.

Table 1. Improved efficency 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™ 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™ 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™ 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%

*Tradtional medium: basal medium supplemented with ESGRO®

Stroma Free Expansion of **CD34⁺ Stem Cells**

In addition to unique applications for murine ES cell culture RESGRO™ Culture Medium can also be used for the stroma-free expansion of adult CD34⁺ umbilical cord blood (UCB) cells.

The potential of RESGRO™ Culture Medium to expand purified human CD34⁺ UCB cells on bare culture plates was compared to UCB cells cultured on a murine feeder cell layer (AFT024). Both culture systems were cultured with or without a cocktail of early acting cytokines (20 ng/ml human IL-6, 200 ng/ml human IL-6 receptor soluble, 20 ng/ml human Flt-3 Ligand and 20 ng/mL human TPO).

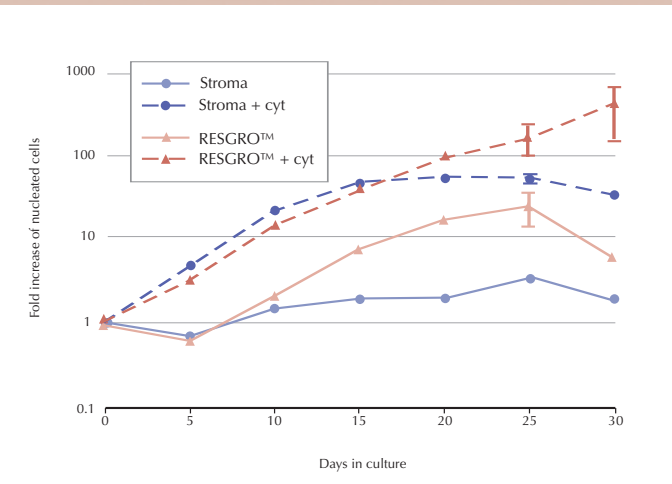


Figure 1. Proliferation of nucleated cells from CD34⁺ UCB cultured either on stroma layer or on bare dishes with RESGRO™ media ± cytokines. The blue trypan exclusion method was used to determine the total viable cell content of expanded cultures. The starting population was represented by 10^5 UCB CD34⁺ cells. n=9, =p<0.001, cytokines cocktail= rh IL-6/rh IL-6 sR/h TPO/h Flt-3L.

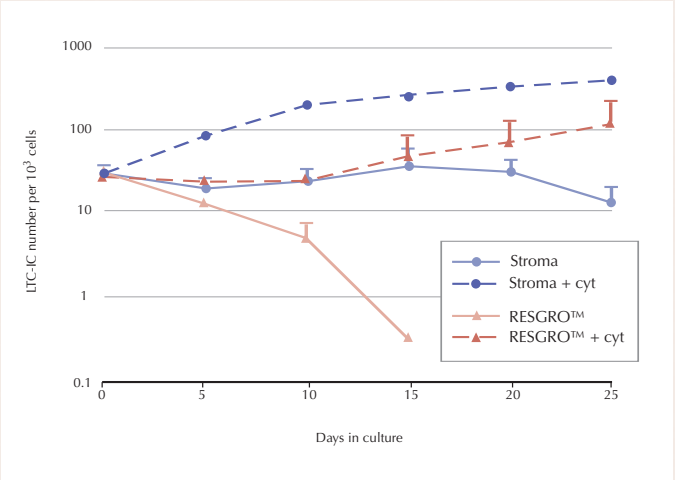


Figure 2. Kinetics of LTC-IC activities in UCB CD34⁺ cultured either on stroma layer or on bare dishes with RESGRO™ media ± cytokines. The frequency of LTC-IC was calculated by using Poisson statistics. n=9, =p<0.01, cytokines cocktail= rh IL-6/rh IL-6 sR/h TPO/h Flt-3L.

Following 30 days culture in RESGRO™ culture medium with cytokines, CD34⁺ UCB cells showed a 200-300 fold increase in the number of nucleated cells compared to cells cultured on stroma (Figure 1). Furthermore, expansion cultures at 3 weeks after initiation in RESGRO™ medium + cytokines consisted of predominantly long-term progenitors (LTC-IC), with a three-fold increase over control cultures recorded (n=9, $p<0.01$ versus baseline, Figure 2). For comparison, the traditional stroma + cytokines system induced a 10-fold expansion of CD34⁺ cells. The maintenance of hematopoietic proliferative potential of cells cultured in RESGRO™ + cytokines on bare culture plates for 5–12 days was demonstrated by the successful engraftment of NOD/SCID mice following transplantation.

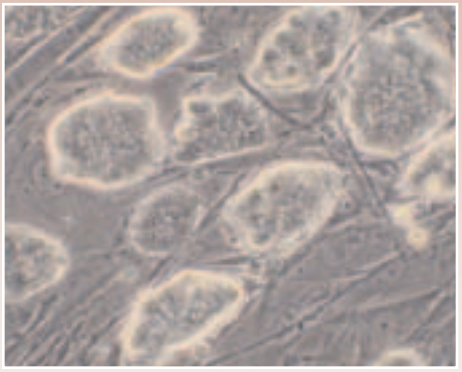
Refer to www.chemicon.com for addtional information on this application of RESGRO™ Culture Medium.

Description	Quantity	Cat. No.
RESGRO™ Culture Medium	250 mL	SCM001
RESGRO™ Culture Medium	500 mL	SCM002
ESGRO®	1 x 10^6 units	ESG1106
ESGRO®	1 x 10^7 units	ESG1107

1. LIF Independant Embryonic Stem Cell Derivation in Mice. Schoonjans L. *et al.* www.chemicon.com

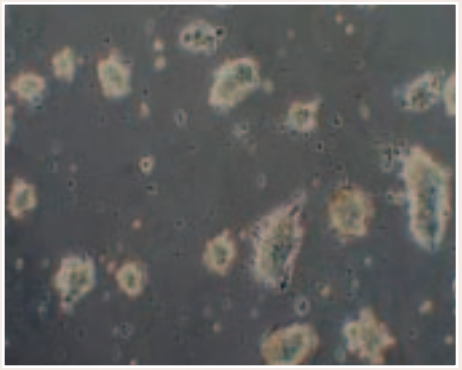
Derivation of **Murine ES Cells**

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.



DBA-2N embryonic stem (ES) cells passage 67 on mouse embryonic fibroblast (MEF) cells.

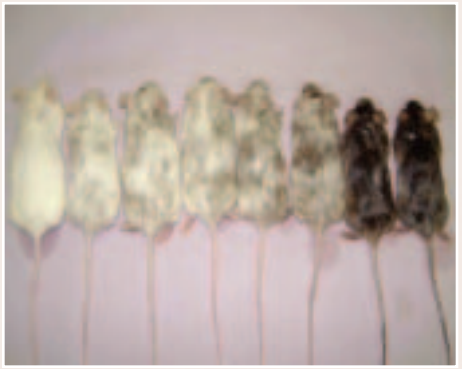
The efficiency of ES cell derivation is greatly strain dependent. To date, very few murine ES cell lines are available from inbred strains other than 129 strains, and those derived have generally been obtained with low success rates. Furthermore, ES cells derived from other strains than 129 are in general 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.



C57/Bl6N ES cells on bare dish.

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™ 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 strains[1].

ES cell lines were derived from all of 5 inbred mouse strains tested, with 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. These chimeras had the capability 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.



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%.

Table 2. Efficiency of ES Cell Derivation and Germilne Competence with RESGRO™ Culture Medium

Mouse Strain	Blastocysts Cultured (n)	Established ES cell lines (n)	(%)	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

Refer to www.chemicon.com for addtional information on this application of RESGRO™ Culture Medium.

CHEMICON®
INTERNATIONAL
a Serologicals® Company

28820 Single Oak Drive
Temecula, CA 92590 USA

PRSRT STD
U.S. POSTAGE
PAID
CHEMICON
INTERNATIONAL