ESGRO Complete™ Clonal Grade Medium: Serum-Free Medium for Mouse Embryonic Stem Cell Culture

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Abstract

ESGRO Complete Clonal Grade Medium is a fully defined, serum-free media for mouse ES cell culture. It is equivalent to serum-containing media for the maintenance of murine embryonic stem cells in the pluripotent state, even in feeder-free culture. ESGRO Complete Clonal Grade Medium is suitable for all standard transgenic protocols from single-cell cloning to the efficient generation of chimeric animals, and thus can replace the need for serum-containing media for multiple mouse ES cell applications. Also noteworthy are the utility of two additional reagents for serum-free culture of murine ES cells, ESGRO Complete Accutase™ for cell dissociation and ESGRO Complete Freezing Medium for serum-free cryopreservation.

Introduction

Fetal bovine serum (FBS) has remained an indispensable component of standard culture media for murine embryonic stem cells, despite several drawbacks. First, FBS is very expensive, and may not always be available. Second, lot-to-lot variability in the performance of murine ES cells is common with FBS; therefore, multiple lots of FBS must be pre-tested prior to purchase of each new lot. Finally, the composition of FBS is unknown; it is certainly preferable that any media used for ES cell culture be completely defined.

Here we introduce ESGRO Complete Clonal Grade Medium, a serum-free and fully defined media for multiple applications of mouse ES cell culture. We show that mouse ES cells cultured for multiple passages remain pluripotent and maintain a normal karyotype. In addition, ESGRO Complete Clonal Grade Medium is superior to standard FBS-containing media for the generation of single mouse ES cell clones, particularly in that pluripotent clones arise earlier than with media containing FBS. Finally, mouse ES cells cultured in this medium yield high-percentage chimeras. Thus, ESGRO Complete Clonal Grade Medium is suitable for multiple applications of mouse ES cell culture, and bypasses the need for FBS.

Results

Murine ES cells [(strains E14Tg2a.IV (hereafter referred to as "E14") originally at passage 16, and 129/S6, originally from Specialty Media at passage 11)] were maintained without a feeder layer in gelatinized tissue culture dishes in 7.5% CO_2. Cells were grown in parallel cultures in either ESGRO Complete Clonal Grade Medium or standard mouse ES cell media (with 15% FBS, prescreened for optimal growth, HyClone) supplemented with nonessential amino acids, sodium pyruvate, 2-mercapto-ethanol and 1000 U/mL recombinant murine LIF (ESGRO®, Millipore Corporation). Media was changed once per day. Cells were maintained at standard densities (i.e., between $5 \times 10^4/cm^2$ and $5 \times 10^5/cm^2$), and were passaged every 2-3 days with ESGRO Complete Accutase (Millipore Corporation), a dissociation reagent that does not require quenching with serum.

After 10 successive passages in either media, cells in culture were examined for their morphology and pluripotency. Cultures of E14 had normal morphology (Figure 1A); most cells were in rounded clusters, with scattered individual cells (often larger than cells in clusters and with a flattened shape) appearing between clusters. This morphology is typical of mouse ES cells cultured without a feeder layer, and was also observed with the 129/S6 cells, as well as with either strain cultured in standard FBS-containing media (not shown).

Mouse ES cells that had been cultured for 10 successive passages in ESGRO Complete Clonal Grade Medium were also examined for expression of pluripotency markers. E14 cells maintained expression of alkaline phosphatase[1], as determined by colorimetric activity staining (Figure 1B). In addition, E14 cells maintained expression of the pluripotency marker Oct4[2], as shown by indirect immunofluorescence for Oct4 protein (Figure 1C, D). Similar results were observed with 129/S6 cells, as well as with either strain in standard FBS-containing media (not shown).

Cells were examined for chromosomal abnormalities after 12 successive passage. in ESGRO Complete Clonal Grade Medium. Karyotype analysis (GTW banding method) demonstrated a normal karyotype (data not shown).

To examine the suitability of ESGRO Complete Clonal Grade Medium for the generation of single mouse ES cell clones (such as for clone selection during standard murine transgenic protocol), cells of either the E14 or 129/S6 strains were plated in either ESGRO Complete Clonal Grade Medium or standard FBS-containing media at a density of 25 cells/cm^2 (on gelatinized TC plastic; note that cells plated into a particular media had already been passaged 5 times at standard densities in that media). Media was changed daily beginning the third day after plating. By day 4 (day 0 = plating), colonies were already visible in the ESGRO Complete Clonal Grade Medium for both strains, and by day 6 these colonies were of a sufficient size to be picked; these colonies were composed of undifferentiated cells, as shown by alkaline phosphatase activity staining (Figure 2A). In contrast, colonies in standard

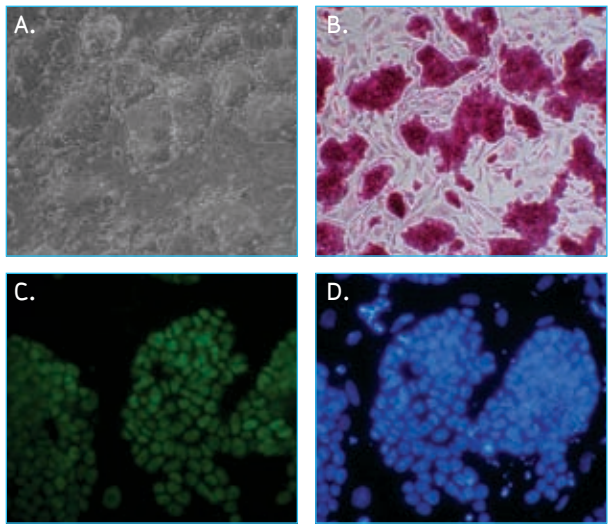

Figure 1. Murine ES cells passaged with ESGRO Complete Clonal Grade Medium maintain normal morphology and expression of pluripotency markers. All cells are E14Tg2a.IV that have undergone 10 successive passages. (A) ES cells appear morphologically normal. Most cells are in rounded clusters, and look typical of mouse ES cells cultured without a feeder layer. (B) Cells in rounded clusters express alkaline phosphatase, a marker for pluripotency. (C), (D) Cells in rounded clusters express Oct-4 (C), another pluripotency marker, as shown by indirect immunofluorscence for the Oct-4 protein (arrow denotes isolated cells in (D), revealed by DAPI staining, that do not express Oct-4).

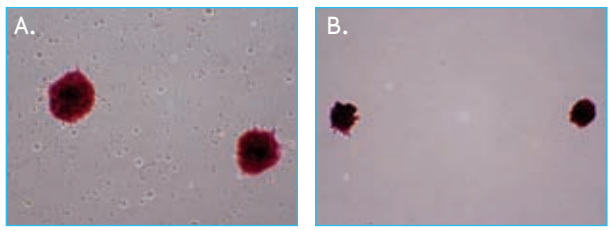

Figure 2. ES cells grown at clonal density in ESGRO Complete Clone Grade Medium yield pluripotent colonies more rapidly than FBS-containing media. (A) E14Tg2a.IV colonies grown in ESGRO Complete Clone Grade Medium at day 6, labeled for alkaline phosphatase activity. (B) E14Tg2a. IV colonies grown in standard 15% FBS media at day 9, labeled for alkaline phosphatase activity. Note that the colonies in (B) are smaller than those in (A), even with three additional days of culture.

FBS-containing media were not easily observed until day 6, and were not of a sufficient size until day 9 or 10; these colonies also contained pluripotent cells (Figure 2B). Thus, not only is ESGRO Complete Clonal Grade Medium suitable for single-clone generation, but pluripotent clones arise more rapidly in ESGRO Complete Clonal Grade Medium than in standard FBS-containing media.

To examine the competency of mouse ES cells cultured in ESGRO Complete Clonal Grade Medium for the generation of transgenic mice, E14 cells that had gone through 11 passage. (and were therefore at passage 27) were injected into mouse blastocysts, and the resulting chimeras examined (Table 1); it should be noted that between passages 10 and 11, these cells were cryopreserved in ESGRO Complete Freezing Medium, which is also serum- free, and subsequently thawed for injection. 40 blastocysts injected (16 live births) resulted in 6 males that were greater than 85% chimeric by coat color, indicating that mouse ES cells cultured in ESGRO Complete Clonal Grade Medium can successfully contribute to adult tissues, and result in the efficient generation of chimeric animals.

Discussion

Here we have demonstrated the utility of ESGRO Complete Clonal Grade Medium, a serum-free, fully defined media for the culture of mouse embryonic stem cells. This medium is suitable for multiple applications involving mouse ES cells,

Blastocytes Injected	Live Births	% Male Chimeras
40	16 (40%)	6 (15%)

Table 1. Generation of High-Percentage Male Chimeras from E14Tg2a. IV cells cultured for 11 passages with ESGRO Complete Clonal Grade Medium.

including general culture at standard densities, single-cell cloning and generation of transgenic mice. Mouse ES cells cultured in ESGRO Complete Clonal Grade Medium, maintain their pluripotency as well as normal karyotype and the capacity to differentiate. ESGRO Complete Clonal Grade Medium performs as well as, or superior to, serum-containing media in all applications tested.

The use of ESGRO Complete Clonal Grade Medium thus bypasses the need for FBS for mouse ES cell culture. This provides the user with several benefits, including being able to avoid the expense and the lot-to-lot variability of FBS as well as the effort necessary to qualify media prior to purchase. Finally, it should be noted that two additional Chemicon products, ESGRO Complete Accutase and ESGRO Complete Freezing Medium, are particularly suited to serum-free mouse ES cell culture.

References

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2. Nichols, J., Zevnik, B., *et al, Cell* **95**: 379-391 (1998).

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