Feeder-free and Serum-free ES Cell Culture using ESGRO Complete System

The ESGRO Complete cell culture system is a fully defined, serum- and feeder-free system based on the work of Ying, *et al*. (2003). The cornerstone of this system is the ESGRO Complete Clonal Grade Medium, a defined serum-free medium optimized to grow and maintain undifferentiated mouse embryonic stem cells in the absence of serum and feeder cells. The mouse ES cells should maintain germ line competency.

The following protocol is applicable for adapting both feeder-dependent and feeder-independent mouse ES cells to serum-free cell culture conditions. It is important to pre-warm all reagents to 37 °C prior to use and avoid using glassware, as ES cells in serum-free media are sensitive to any residual detergent. The use of disposable plasticware in any manipulations is strongly recommended.

Materials & Reagents required:

- Clonal Grade Medium (Cat. No. SF001-500)
- Basal Medium (Cat. No. SF002-500)
- Accutase™ Solution (Cat. No. SF006)
- Gelatin Solution (Cat. No. SF008)
- DPBS (Cat. No. BSS-1006-C)
- T25 Flasks
- Incubator, 37 °C

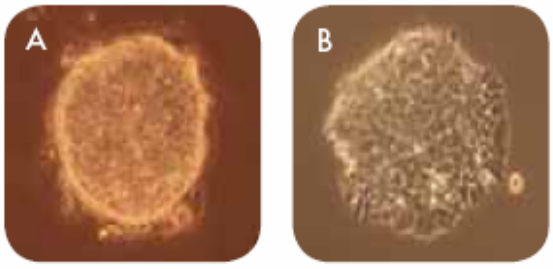
Procedure:

1. Grow mouse ES cells to 60% confluence in serum-supplemented medium in a T25 flask with or without feeders.
2. Change medium 24 hours prior to seeding into serum free conditions.
3. Pre-coat T25 flasks with Gelatin Solution.
4. Wash cells once with DPBS. To dissociate cells, add 1 mL Accutase solution. Incubate at 37 °C and allow cells to detach (5–10 minutes). Tap gently and add 5 mL of Basal Medium, mix and spin at 1000 rpm.
   Note: It is important not to use standard trypsin. “Balling” of ES cells can occur (see Image 6C).

5. Remove supernatant and resuspend pellet in 5 mL Clonal Grade Medium. Count cells.
6. Plate 1x10^6 cells into a pre-coated T25 flask containing 10 mL pre-warmed Clonal Grade Medium.
7. Observe cell growth over the next 2–3 days. Some residual feeders may remain stuck down, some cell death may be observed and some differentiation may be visible.
8. However, ES cells colonies will continue to grow and may appear to be flatter than those on feeders with a distinct nuclear and cytoplasmic morphology (See Images 6A and 6B).
9. When mES cells are about 60% confluent, perform a 1 in 5 split of the T25 flask into another coated T25 flask containing Clonal Grade Medium. Note: Do not let ES cells in serum free media become over confluent as they will begin to differentiate.
10. To passage the ES cells, remove the media and wash with DPBS buffer. Add 1 mL of Accutase solution per T25 flask. *Note: Do not use standard trypsin.*
11. Return to 37 °C incubator and allow cells to detach (5–10 minutes). Tap gently and add 5 mL Basal Medium; mix and spin at 1000 rpm.
12. Remove supernatant. Resuspend pellet in 5 mL Clonal Grade Medium.
13. Cell growth should be observed over the next 2–3 days. The ES cells should be passaged as in steps 9 and 10 an additional 2–3 times. After these passages no or few residual feeders should remain and the remaining ES cells should have only low levels of differentiation (see Image 6D).

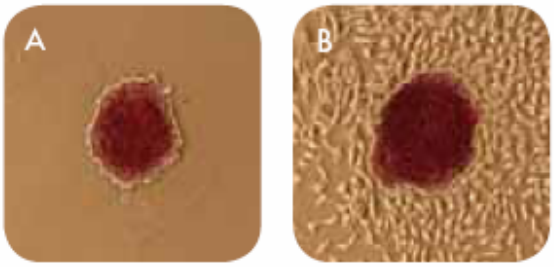
Ying, Q., Nichols, J., Chambers, I., Smith, A. (2003). BMP Induction of Id Proteins Suppresses Differentiation and Sustains Embryonic Stem Cell Self-Renewal in Collaboration with STAT3. Cell 115:281-292.

Image 6A: Undifferentiated ES cells colonies with a distinct cytoplasmic and nuclear morphology (day 4 of a clonal assay).



(A) Tight round colony (B) flatter colony

Image 6B: Alkaline phosphatase staining allows for an easy distinction between undifferentiated ES cells (red) and differentiated cells (unstained) on day 5 of the clonal assay.



(A) ES cells colony with no differentiation

(B) differentiated cells at the edge of an ES cells colony

Image 6C: Standard Trypsin use causes the ES cells to lift off the plates in serum free conditions (A) whereas the use of Accutase solution allows for an efficient and gentle way to routinely passage the cells (B).

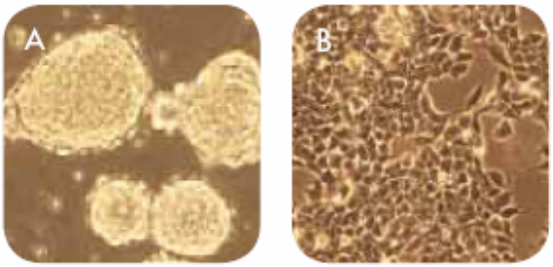


Image 6C: Standard Trypsin use causes the ES cells to lift off the plates in serum free conditions (A) whereas the use of Accutase solution allows for an efficient and gentle way to routinely passage the cells (B).

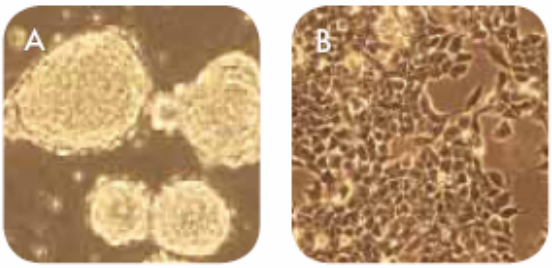


Image 6D: Immunostaining of mouse ES cells grown in Clonal Grade Medium. From left to right for the top panel (A): phase contrast, Hoechst staining and Oct-4 staining. From left to right for the bottom panel (B): phase contrast, Hoechst staining and Nanog staining.