



Selection of ES Cells

Prior to selection, it is recommended that a kill curve should be determined for each ES cell line in order to determine the exact drug concentration to be used. The following selection regimes can be used as a guide:

- Neomycin (G418) in 129SVEV and 129SVJ cells: 350 µg/mL for two days, then 150 µg/mL for the rest of the selection. Total 5–7 days selection.
- Neomycin (G418) in C57BL6/J cells: 275 µg/mL for one day, 200 µg/mL for the second day, then 150 µg/mL for the rest of the selection. Total 6–8 days selection.
- Hygromycin B in 129SVEV and 129SVJ cells: 100 µg/mL for two days, then 75 µg/mL for one day, then 50 µg/mL for the rest of the selection. Total 7–10 days selection.

Materials & Reagents required:

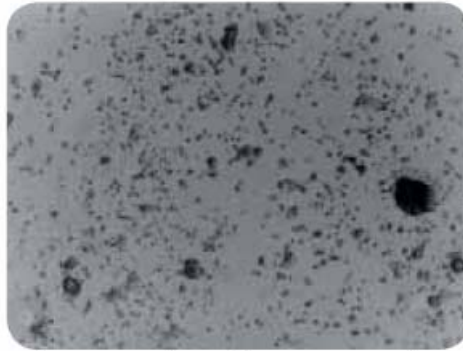
- ES Cell Medium supplemented with either Neomycin (G418) or Hygromycin B
- ES Cell Medium:
 - DMEM (Cat. No. SLM-220-B)
 - 15-20% Fetal Bovine Serum (Cat. No. ES-009-B or ES-011-B)
 - 1% Nucleosides, 100x (Cat. No. ES-008-D)
 - 1% Penicillin-Streptomycin, 100x (Cat. No. TMS-AB2-C)
 - 1% Non-Essential Amino Acids, 100x (Cat. No. TMS-001-C)
 - 1% L-Glutamine Solution, 100x (Cat. No. TMS-002-C)
 - 1% 2-Mercaptoethanol, 100x (Cat. No. ES-007-E)
 - 1000 units/mL ESGRO mLIF Supplement (Cat. No. ESG1106 or ESG1107)
- ES Cell Qualified DPBS (Cat. No. BSS-1006-A, Cat. No. BSS-1006-B)
- Incubator, 37 °C/5% CO₂
- Pipette

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Procedure:

1. To select for transformants, add ES cell medium containing either Neomycin (G418) or Hygromycin B.
2. After 48 hours, cell death should be apparent (see Image 8A). Change the culture medium daily if there is excessive debris evident or if the medium is discoloring, otherwise every other day is sufficient. If the debris is adhering to the living cells, wash the cells gently with sterile DPBS before changing the medium, taking care not to dislodge the feeder cell layer.
3. ES cells should be cultured for approximately 10 days following electroporation.

Image 8A



Electroporated ES cells (R1) grown in the presence of PMEFs and selected with G418 after 5 days of culture (10x).