

VGB6-DERIVED KOMP (REGENERON) CLONES

Cell Line Information

VGB6-derived KOMP clones from Regeneron Pharmaceuticals Inc. are feeder-dependent.

Parental ES cells: VGB6 (formerly B6A6), isolated from C57BL/6NTac mouse strain (Taconic). Injection of these cells into Albino C57BL/6 blastocysts will produce black chimeras. Alternatively chimeras can be also produced by aggregation of ES cells with 8-cell stage embryos from ICR strain or by injection of ES cells into 8-cell embryos from either albino C57BL/6 or Swiss Webster strain. The parental cell line was derived at Regeneron and is certified *Mycoplasma Sp.* free.

Feeder cells: SNL 76/7 mouse embryonic fibroblast (MEF) cells express very small amounts of Leukemia Inhibitory Factor (LIF) and neomycin phosphotransferase (*Neo*). In addition, MEF feeder cells have been derived at UC Davis and Regeneron has approved the use of these feeders for their cell lines. Please see the Mouse Biology Program website (<http://www.mousebiology.org>) for details on using feeder cells.

Reagents and Supplies

VGB6 Medium

Reagent	Suggested Vendor (Catalog Number)	Volume (for 600 mls)
Knockout DMEM	Gibco (10829-018)	479 mls
Knockout Serum Replacement*	Gibco (10828-028)	90 mls
L-Glutamine (200mM)	Gibco (25030-081)	12 mls
Pen-Strep	Gibco (15140-122)	6 mls
Non-Essential Amino Acid Solution	Gibco (11140-050)	6 mls
Sodium Pyruvate (100mM)	Gibco (11360-070)	6 mls
2-B-mercaptoethanol	Gibco (21985-023)	1.2 mls
LIF (2000 u/ml)	Chemicon (ESG1107)	12 ul
Insulin (bovine pancreas, 10 mg/ml)	Sigma (10516)	250 ul

*or ES Cell Grade FBS may be used, 90 mls

Other Reagents

Reagent	Suggested Vendor (Catalog Number)
PBS (1X without Ca or Mg)	Gibco (14190-144)
Trypsin-EDTA (0.25%)	Gibco (25200-072)
DMSO	Sigma (D2650)
Hepes-buffered DMEM for Microinjection media	Gibco (12430-054)

10% (1X) Freezing Medium

To make 20 ml:

- 18 ml of 25% FBS Medium (add 2 ml extra FBS to 16 ml Basic ES Cell medium)
- 2 ml DMSO

Sterile filter through 0.2µM filter

Microinjection Medium

To make 500 ml:

- 475 ml Hepes-buffered DMEM
- 25 ml FBS

Sterile filter through 0.2 µM filter

May be stored at -20 to -80 for up to 1 year

Thawing VGB6-derived KOMP (REGENERON) Clones

1. Thaw 1 vial of p3 or p4 ES cells (approximately $1.5-2 \times 10^6$ cells/vial) in a 37°C water bath and dilute into 10 ml of pre-warmed ES cell medium.
2. Pellet the cells by spinning for 5 minutes at 1,200 rpm.
3. Aspirate off medium and gently resuspend cells in 4 ml of pre-warmed ES cell medium.
4. Aspirate the old MEF medium from your 6-well plate containing inactivated MEF Feeder cells.
5. Split the ES cell suspension between the duplicate wells of your 6-well feeder dish, and grow in a 37°C humidified 5% CO₂ incubator.

6. Change medium the following day to remove dead cells and residual DMSO.
7. Two days after thawing change medium in both wells in the morning.
8. If they've reached 40-60% confluency, cells from one well can be trypsinized 3-4 hour later for microinjection
9. Next day, cells in the duplicate 6-wells should reach 70-80% and may be frozen into 2-3 vials.

Freezing VGB6-derived KOMP (REGENERON) Clones

1. 3-4 hours prior to freezing, refeed the 70-80% confluent well of the 6-well plate 3 ml pre-warmed ES cell medium (VERY IMPORTANT.)
2. After the 3-4 hours; aspirate off the media, and rinse once with 2-3 ml PBS.
3. Aspirate PBS and overlay the cells with 0.5 ml of 0.25% trypsin solution and incubate at 37°C for 10 minutes.
4. Add 2.5 ml ES cell medium to inactivate the trypsin, and pipette very gently to make single cell suspension (we recommend 3-4 times only.)
5. Transfer to 5 or 10 ml tube and spin for 5 minutes at 1,200 rpm.
6. Aspirate supernatant and resuspend the pellet in 1 ml of the 1X freezing medium.
7. Decant the cell suspension into 2-3 labeled cryovials.
8. Immediately place cryovials in a Styrofoam container or temperature controlled freezing vessel.
9. Freeze vials in a -80°C freezer. After 24 hours, transfer cryovials to liquid or vapor-phase nitrogen for longer term storage.

Thawing of VGB6-Derived KOMP (REGENERON) Clones for Micro-Injection

- 1.** 1 day prior, prepare one 24 well plate with inactivated MEF Feeder cells. (4 well per clone to be thawed)
- 2.** Next day thaw a vial previously frozen ES cells in a 37°C water bath and dilute into 5 ml of pre-warmed ES cell medium.
- 3.** Pellet the cells by spinning for 5 minutes at 1,200 rpm.
- 4.** Aspirate off medium and gently resuspend cells in 1.2 ml of pre-warmed ES cell medium.
- 5.** Plate into each well of a 24-well feeder plate, the following dilutions of cell suspension:
 - 100µl, 200µl, 300µl, 500µl
- 6.** Change medium the following day to remove dead cells and residual DMSO.
- 7.** Following day examine all wells, and determine which dilution is optimum for microinjection (confluency should be 40-60%, and morphology should be bright, small, smooth and round colonies.)
For the optimum cell dilution, trypsinize as standard, spin and resuspend the cell pellet in Microinjection Medium. Immediately place the tube of cells on ice, and microinject within 1-2.5 hours.
- 8.** Change media in the remaining wells, cells can be used for microinjection over next 2 days if satisfy the same criteria as the first day (confluency should be 40-60%, and morphology should be bright, small, smooth and round colonies.)