

NHE Null (Li poisoning and selection method) Generation Protocol

Part One – Mutagenesis (Proc. Natl. Acad. Sci. USA Vol. 77, No. 5, pp. 2698-2701, May 1980)

- ✓ Plate cells into four T-25 flasks and culture until growing exponentially (~ 30 – 50% confluent or $1-2 \times 10^4$ cells per cm^2)
- ✓ Treat with filter sterilized ethyl methanesulfonate (EtMes) at 0.25 $\mu\text{l}/\text{ml}$ in complete medium for 16 hr.
 - *In these conditions, 50% of the cell population should survive.*
- ✓ The next day, wash the cells twice with PBS, and continue to culture, 2-6 days (*until cells are 50-60% confluent*) in T-25 flasks before selection. Split cells 1:2 keeping all flasks when 70-80% confluent.

Step Two – Li Poison Selection (Proc. Natl. Acad. Sci. USA Vol. 81, pp. 4833-4837, August 1984)

Start with an exponentially growing culture,

- ✓ Trypsinize cells from each T-25 cultured in step one and add 5 ml of complete media, centrifuge at 300 rpm for 5 min and aseptically remove media. Keep identification numbers for each flask.
- ✓ Resuspend the cells in 5 ml of LiCl saline solution in a 15 ml falcon tube.
- ✓ Incubate for 2 hrs at 37°C
- ✓ Centrifuge cells at 300 rpm for 5 min.
- ✓ Remove solution and resuspend pellet in 10 ml of choline CL acid saline solution.
- ✓ Centrifuge again and remove solution. Resuspend cells in 5 ml of choline CL acid saline solution.
- ✓ Incubate for 60 min including centrifugation time.
- ✓ Pellet cells again by centrifugation and immediately resuspend cells in 1 **T25** with complete media.
- ✓ After 2-4 days (**when cells are at 50% confluence**) repeat the Li/Acid loading cycle

- ✓ When, after the second round of selection cells are 30-50% confluent, select for individual colonies using the "single cell cloning by serial dilution" method by Corning. Each T-25 will be used for two or more plates.

LiCl saline solution: 130 mM LiCl
5 mM KCl
1 mM MgSO_4
2 mM CaCl_2
5 mM glucose
20 mM HEPES-Tris, pH 7.4.

Choline Cl acid saline solution: 130 mM choline Cl
5 mM KCl
1 mM MgSO_4
2 mM CaCl_2
20 mM 2-(N-morpholino)ethanesulfonic acid-Tris, pH 5.5.