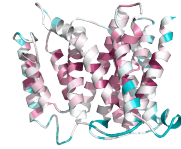


Li⁺ Poison and Selection Protocol



PROTOCOL

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

1. 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)
2. Treat with filter sterilized ethyl methanesulfonate (EtMes) at 0.25 $\mu\text{l/ml}$ in complete medium for 16 hr.
 - a. *In these conditions, 50% of the cell population should survive.*
3. 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.

1. 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.
2. Resuspend the cells in 5 ml of LiCl saline solution in a 15 ml falcon tube.
3. Incubate for 2 hrs at 37°C.
4. Centrifuge cells at 300 rpm for 5 min.
5. Remove solution and resuspend pellet in 10 ml of choline CL acid saline solution.
6. Centrifuge again and remove solution. Resuspend cells in 5 ml of choline CL acid saline solution.
7. Incubate for 60 min including centrifugation time.
8. Pellet cells again by centrifugation and immediately resuspend cells in 1 T25 with complete media.
9. After 2-4 days (**when cells are at 50% confluence**) repeat the Li/Acid loading cycle
10. 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.

SOLUTION

LiCl Saline Solution:	Choline Cl Acid Saline Solution:
130 mM LiCl	130 mM Choline Cl
5 mM KCl	5 mM KCl
1 mM MgSO ₄	1 mM MgSO ₄
2 mM CaCl ₂	2 mM CaCl ₂
5 mM glucose	20 mM 2-(N-morpholino)ethanesulfonic acid
20 mM Hepes-Tris	MES pH 5.5.
pH 7.4.	