

EL-4.B5 Freezing medium

Make fresh for each use.

90% HI-FCS

10% DMSO, Sigma #D2650

Sterilely add reagents together, and mix thoroughly. Pre-warm before use.

Medium may be sterile filtered w/ 0.2um PES filter unit (Nalgene #565-0020), if desired.

EL-4.B5 Freezing Protocol

1. Count log-phase EL-4.B5.
2. Centrifuge cell suspension @ 1700rpm, 5min, RT.
3. Gently resuspend cell pellet in freezing medium @ a density of 2×10^6 EL-4.B5/mL.
4. Distribute one mL aliquots into pre-labeled 2mL screw-capped, o-ring cryovials (Sarstedt #72.694.996).
5. Place filled cryovials into a freshly charged Mr. Frosty freezing container (Nalgene #5100), and place @ -70°C overnight.
6. Next day, cryovials may be moved to vapor phase of a liquid nitrogen freezer for long-term storage.

*Alternately, filled cryovials may be placed directly into a liquid nitrogen freezer, but with a minimum 24-hour vapor phase period **before** moving to the liquid phase.*