



## LM105 – Cell Recovery After Shipping

**These recommendations are for recovering hybridoma cells after shipping at room temperature in culture media.**

Cells were grown in hybridoma cell culture media (DMEM, 10% FBS, Pen-Strep) until cell density reached  $1.5-2 \times 10^5$  cell/ml for shipping. Sterile 15-ml conical tubes were filled up with cell culture (total  $2.25-3 \times 10^6$  cells), capped tight and sealed with Parafilm, shipped at room temperature. For recovery, follow this protocol:

- a) Centrifuge cells at 1000 rpm (about 170 g) in Beckman centrifuge for 5 min.
- b) Remove supernatant (conditional medium) to a new tube.
- c) Gently flick the tube containing the cells a few times to resuspend.
- d) If cell pellet is too large to be resuspended, add 3-4 ml of the conditional medium back if not acidic (yellow) and transfer cells in a small TC flask (T25). If the conditional medium has turned acidic, add half conditional medium and half fresh medium.
- e) For fewer cells, add less media. Store remaining conditional medium at 4°C as it can be used for splitting the cells.
- f) Incubate the cells in a 5% CO<sub>2</sub>/37°C incubator and wait for recovery (2-3 days). Do not shake the cells often before they have been fully recovered.
- g) When changing medium, the conditional medium can be added before changing to fresh medium.
- h) Split cells 1:3 every 2-3 days when media turns orange/yellow, transferring cells into larger flasks when appropriate.

### Notes:

- The cells are maintained in DMEM, 10% FCS, 100 u/ml penicillin and 100 µg/ml streptomycin (1µg/unit).
- Work in sterile conditions.
- Hybridoma cells can stand acidic conditions (yellow) but not basic conditions (purple medium).