# 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 x $10^5$ cell/ml for shipping. Sterile 15-ml conical tubes were filled up with cell culture (total 2.25-3 x $10^6$ 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% CO2/37°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).