



**Title:** *Cell Culture: Subculturing of Cells*

No: RTLP-GL-CP-12

Location:  
*Old CCRC Tripp Lab*

Approval Date:  
10 September 2004

Supersedes Date:

**Materials:**

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- |                        |                           |                 |                  |
|------------------------|---------------------------|-----------------|------------------|
| •Lab coat              | •DMEM                     | •Tissue culture | •Pipettes        |
| •Gloves                | •37°C, 5% CO <sub>2</sub> | flasks          | •Pipetteman      |
| •0.5% trypsin-EDTA/4Na | incubator                 | •Centrifuge     | •Pipette Aid     |
| • Fetal bovine serum   |                           |                 | •Pipetteman tips |

**Procedure:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.05% Trypsin, with EDTA/4Na solution (Gibco, Cat# 25300-062) to remove all traces of serum which contains trypsin inhibitor.
3. Add 7.5 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Incubate cells at 37°C for 5-10 minutes to dissociate cells.
4. Add 7.5 ml of complete growth medium (Dulbecco's modified Eagle medium (Gibco, Cat# 25300-062) supplemented with 10% fetal bovine serum (Hyclone, Cat # 30070.03) and aspirate cells by pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C, 5% CO<sub>2</sub>.