



Fibroblast Cell Culture

1. The goal of the procedure is to establish fibroblasts for cell culture applications.
2. If the cells are being subcultured, then grow them as outlined in the following steps.
If samples are from frozen cells refer to the protocol for thawing cells and continue using the cell culture step in the present procedure.
3. Make sure to clean Biosafety cabinet and all containers and reagents with 70% Isopropanol.
4. Cells are ready for subculture when they have reached a confluence of 80-90% in a monolayer.
5. Warm up the Culture Medium (DMEM/10%FBS) at 37°C for about 10-15 minutes.
6. Warm up Trypsin (0.05%) at room temperature for 15-30 minutes.
7. From a flask/dish containing cells, aspirate cell culture medium and discard medium.
8. Add HBSS(-) or DPBS(-)—depending on the size of culture flask/dish. Add enough to cover all cells and to wash out the remaining medium because they can interfere with Trypsin action when added.
9. To make sure that the cells are washed properly, make sure to swirl the flasks or dishes in a circular motion to cover all areas.
10. Aspirate the HBSS(-) or DPBS(-) from each dish or flask and discard buffer. Repeat wash if necessary.
11. Add Trypsin according to flask or dish being used. In general use 0.64-0.1 mL/cm²—make sure to follow the manufacture's recommendations.



<p>12. Incubate the cells at 37°C for about 5-15 minutes (until all cells have detached). Observe under microscope to make sure all cells have lifted from flask/dish.</p>
<p>13. Neutralize the enzymatic activity of Trypsin by adding equal volume of Cell Culture medium.</p>
<p>14. Transfer cells into 15 or 50ml tube using a pipette. Wash sample(s) with HBSS(-) or DPBS(-) buffer 2 times; aspirating buffer with a glass Pasteur pipette.</p>
<p>15. Examine flask or dishes under microscope to make sure all cells were collected from flask/dish.</p>
<p>16. Centrifuge cell suspension at 150g for 5 min at 4°C to pellet cells.</p>
<p>17. Aspirate supernatant carefully and discard supernatant.</p>
<p>18. Re-suspend cells in Cell Culture (depending on the amount of flasks/dishes used) and count cell using a hemocytometer (as described on cell counting protocol).</p>
<p>19. Plate cells at density between 1,000-3,000 cells/cm². Set up the necessary flasks/dishes for each particular experiment or use.</p>
<p>20. Incubate cells in incubator at 37°C and 5% CO₂.</p>
<p>21. Change culture media every 2-3 days. Do a complete media change by removing flasks/dishes from incubator and aspirating old media with fresh warm (at 37°C) Cell Culture media.</p>
<p>22. After feeding cells return flasks/dishes into the incubator.</p>
<p>23. Once cells growing reach a confluency of 80-90%, cells are ready to be passed once again (repeat 2-23). When cultures reach 80%-90% confluency, freeze cells at 1.5 x10⁶cells/vial in respective freezing solution for Cell Banking (see freezing protocol) or continue passing cells as previously described.</p>