Cell culture of 7721 or HeLa cells

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Abstract

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Protocol

Step 1.

Prepare RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Hyclone, USA) and 1% penicillin/streptomycin (Gibco, USA).

Step 2.

Warm medium to 37℃ and bring 0.1% trypsin - ethylenediamine tetraacetic acid (EDTA) to room temperature.

Step 3.

Observe cells under microscope to evaluate cell confluency and presence of cell death.

Step 4.

Aspirate out spent medium when the cells reach approximately 90% confluency, and wash twice with PBS.

Step 5.

Add 1 ml 0.1% trypsin-EDTA to 10-cm culture dish, and ensure the entire surface of the dish is covered with 0.1% trypsin-EDTA. Incubate the cells at 37℃ for 2 minute.

Step 6.

Observe cells under the microscope until the cells have been fully detached, then aspirate out the 0.1% trypsin-EDTA.

Step 7.

Add 5 ml medium to the dish, gently pipet the cells, and harvest the cells by centrifugation at 1,000 rpm for 5 minutes.

Step 8.

Aspirate out the medium, minding to not disrupt the cell pellet.

Step 9.

Resuspend the cells in 2 ml medium and count with trypan blue.

Step 10.

Seed the cells in 24-well plates at a density of $1x10^5$ cells/500 µl. Culture cells under 5% CO_2, in a 95% humidified atmosphere at 37℃.