
SOP NUMBER: 114
TITLE: Trypsinization of Adherent Cells

Principle:

Adherent cells attach themselves to surface of tissue culture flasks or dishes using proteins. These proteins, secreted by the cells, form a tight bridge between the cell and the surface. To dislodge cells from the flask, the protein bridges must be broken. Trypsin is a proteolytic (protein degrading) enzyme that will break proteins at specific places. EDTA is often found in trypsin solutions. EDTA allows trypsin to work more efficiently by engaging certain metal ions that may inhibit its activity.

NOTE 1: Cells must NEVER remain in trypsin for longer than 3-5 minutes.

NOTE 2: At no time should cells be left without a fluid layer.

NOTE 3: Cells that have grown on the flask for more than a week will be the most difficult to remove.

I. Materials:

A. Equipment

Biosafety cabinet
Tabletop centrifuge
Hemocytometer with cover glass
Incubator, dual chamber
Microscope, inverted
Pipet-Aid

B. Supplies

Aspirating pipets: 2 ml
Lab permanent marker (water proof/alcohol proof)
Nitrile gloves
Liquid waste container (containing 10% bleach)
Polystyrene centrifuge tubes with screw cap, 15 ml and 50 ml
Serological Pipettes: Sarstedt: 1ml, 2 ml, 5,10 ml
Timer (or clock)

C. Media and Reagents

Complete media
Dulbecco's Phosphate Buffered Saline (DPBS) w/o calcium and magnesium (1x)
Trypan blue stain 0.4%
0.25% Trypsin-EDTA
0.05% Trypsin-EDTA

II. Method:

Procedures are to be done in the Biological Safety Cabinet using aseptic techniques

1. Label 15 ml or 50 ml conical tube with cell line designation.
2. Remove media from flask using appropriate sized serological pipet.
Note: Adherent cells should remain on the flask surface.
3. Rinse cells with 1-10ml of D-PBS depending on the flask size.
4. Add an appropriate volume of trypsin (see chart below)
5. Incubate for 1-3 minutes at RT (ambient temperature) or 37°C.

SOP NUMBER: 114
TITLE: Trypsinization of Adherent Cells

7. Dislodge cell by rapping flask on surface.
8. Confirm under microscope that cells are off the flask surface.
9. Add media to flask (1-10 ml).
10. Pipet forcefully down lower surface to dislodge remaining cells and to stop the trypsin.
11. Transfer cells to the pre-labeled test tube.
12. Centrifuge at 1500-2000 RPM (150xg) for 15 min. at ambient temperature.
13. In BSC, remove supernatant being careful not to disrupt the cell pellet. This can be done by gentle aspiration or pipetting.
14. Resuspend the cells in D-PBS (1-5ml). Volume will depend on the size of the pellet.
15. Perform cell counts and viability according to protocol.

III. Working Volumes for Trypsinization

Flask Type	Flask Size	Volume of Trypsin
Round Dishes	35 mm	0.2 – 0.3 ml
	60 mm	0.5 – 0.6 ml
	100 mm	1.0 ml
	150 mm	1.5 ml
Multi-well Plates	6-well	0.2 – 0.3 ml
	12-well	0.1 – 0.2 ml
	24-well	0.08 – 0.1 ml
	48-well	0.05 – 0.08 ml
	96-well	0.01 – 0.02 ml
Flasks	T-25	0.5 – 0.8 ml
	T-75	1.0 ml
	T-175	2.0 ml
	T-300	4.0 ml
Roller Bottles	125-400 ml (Single Piece)	10 – 15 ml
	125-4000 ml (3-Piece)	10 – 15 ml
	250-800 ml (3-Piece)	20 ml
	200-400 ml (Pleated Single)	20 ml

The Johns Hopkins Medical Institutions
 The Genetic Resources Core Facility: The Cell Center
 Ballock 1016/600 N. Wolfe Street Baltimore, Maryland 21287
 Director: Margaret B. Penno, Ph.D. Tel: 410-614-0060 E-mail: penno@jhmi.edu
