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Protocol status: Working
We use this protocol and it's working

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Passaging cells in MultiFlasks

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ABSTRACT

Simple protocol for working with Falcon Multi-Flasks

MATERIALS

Falcon 3-layer or 5-layer Multi-Flasks (Corning 353143 or Corning 353144, respectively)

5-layer Multi-Flask

3m

1 Per flask, Prepare and pre-warm:

- 200ml PBS
- 30ml 0.25% Trypsin-EDTA or other detachment reagent
- 70ml complete media for trypsin inactivation, plus extra for reseeding

2 Pour out media in flask, wash out remaining media **twice** by:

1. Adding 100 mL PBS
2. Distributing equally and evenly between layers to cover the entire culture surface area
3. Pouring out PBS

3 Add 30 mL 0.25% Trypsin and distribute equally between layers 3m

Incubate 00:03:00 37 °C

4 Inactivate trypsin with 30 mL media distributed equally between layers

Collect cell suspension into 50ml tubes or 100ml bottles

Wash out remaining cells with 40 mL media

5 Centrifuge at 200 x g, 00:05:00, remove trypsin, resuspend in fresh media if necessary, then coun 5m cells and reseed

6 Per flask, Prepare and pre-warm:

Reagent	2-layer	5-layer
PBS	250ml	500ml
Trypsin	40ml	100ml
Complete media for inactivation	80ml	200ml
Complete media for plating	220ml	550ml