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

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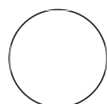
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## Passaging cells in MultiFlasks

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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)

- 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 count 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