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

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Media changes and Passaging in 2- or 5-layer CellStacks

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ABSTRACT

Protocol for handling CellStacks

MATERIALS

Corning CellStacks (2-layer: Corning 3269, 5-layer:Corning 3313)
Complete cell culture media
Phosphate-buffered saline
Trypsin solution (usually 0.25%, with EDTA and phenol red; Gibco 25200)
Centrifuge
50ml centrifuge tubes

BEFORE START INSTRUCTIONS

Ensure shelves of incubator are truly level with a spirit level or app

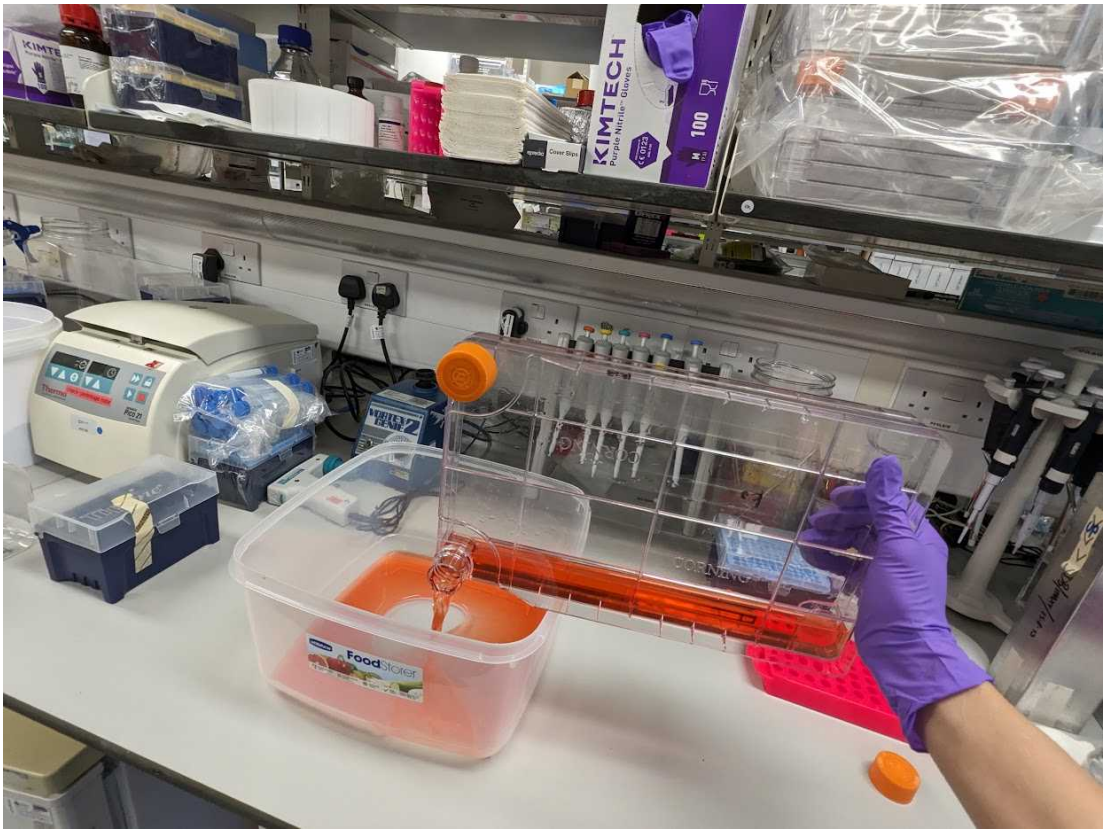
Media Changes

1 Per flask, Prepare and pre-warm:

	Reagent	2-layer	5-layer
	Complete media for plating	220ml	550ml

2 Take flask out of incubator, and place in upright position. Wipe down cap areas with 70% ethanol if desired.

Holding the flask with the long side facing downwards, uncap the bottom cap, and pour out media in flask onto a walls of a large 10L tub to prevent splashing, shake flask to remove the final few drops.

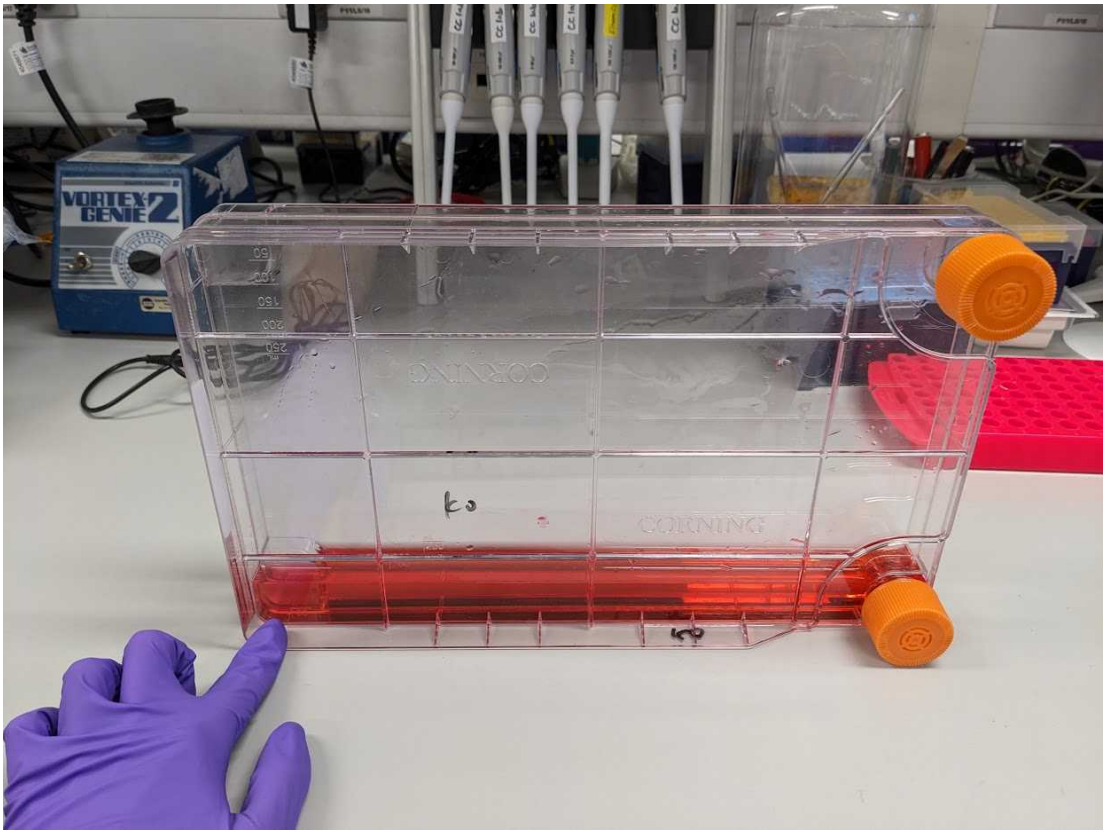


IMPORTANT: Handle flasks for cell culture in a suitable biosafety cabinet. Image taken for demonstration of flask handling only.

3 Place CellStack on its flat position.

For 2-layer CellStacks, pipette in new warm media 50-60ml at a time until 220ml has been added
For 5-layer CellStacks, pour in 550ml of new warm media (often an entire bottle)

4 To evenly distribute media between layers: Flip flask towards you such that it rests on its longer side.



For 2-layer CellStacks, place a finger on the bottom ledge to ensure flask is level.

IMPORTANT: Handle flasks for cell culture in a suitable biosafety cabinet. Image taken for demonstration of flask handling only.

Then tilt into the upright position:

5 Return flask to incubator, lying flasks flat while minimising sudden or large movements to avoid media spilling between layers

Passaging

8m

6 Per flask, Prepare and pre-warm:

Reagent	2-layer	5-layer

	Reagent	2-layer	5-layer
	PBS	250ml	500ml
	Trypsin	40ml	100ml
	Complete media for trypsin inactivation	40ml x2	100ml x2
	Complete media for plating	220ml	550ml

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

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

8 Add Trypsin 3m

Incubate flask for around 00:03:00 in incubator

Tap flask to dislodge cells

9 Add 1 aliquot of media for trypsin inactivation and distribute evenly between layers.
Collect cell suspension into duran bottles (250ml for 2-layer, 500ml for 5-layer)
Wash out remaining cells with the other aliquot of inactivation media similarly.

Reduce clumping by pipetting up and down multiple times with a 10ml serological pipette against the bottom of the bottle

10 Count cells using a Vi-Cell and calculate volume of cells for seeding

11 If necessary, centrifuge cells for replating at 200 x g, 00:05:00 and remove supernatant. 5m

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If plating into CellStacks:

1. Resuspend cells in media for plating
2. Transfer suspension into flask by pouring or pipetting

12 Reseed cells at desired density.

If plating into CellStacks:

1. Calculate the required volume of cell suspension for plating
2. Remove the same volume of media from the pre-warmed aliquot of media for plating and replace with

the cell suspension

3. Transfer suspension into flask by pouring or pipetting

Pelleting cells

5m

13 Transfer desired number of cells (usually 5e7) into 50ml centrifuge tubes

14 Centrifuge cells at 200 x g, 00:05:00 and remove supernatant. 5m
Resuspend with 5 mL PBS , centrifuge and remove supernatant again.

15 Flick tube to loosen pellet and freeze on dry ice.