Creighton UNIVERSITY

Subculture of Adherent Cells

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Equipment:

Pipettes (sterile)
Tissue culture flask (25 cm^2 or other size; sterile)

Reagents:

Media without serum (eg. RPMI; sterile)
Media with serum (eg. RF10; sterile)
Trypsin solution (0.25% in media without serum)
or
EDTA solution (1 mM EDTA)

Method:

1) Remove the old media from the flask.
2) Wash the flask with media without serum.
   - Add 1/2 volume of media without serum to the side of the flask opposite the cells.
   - Tip the media onto the cells to rinse them.
   - Remove and discard the rinse.
3) Add trypsin solution to the flask.
   - Add 3-5ml trypsin to the side of the flask opposite the cells (3-5ml / 25cm^2 flask).
   - Tip the trypsin onto the cells.
   - Leave the trypsin on the cells for approximately 15-30 seconds.
   - Remove and discard the trypsin.
4) Allow the flask to incubate until the cells "round up". Usually 5-15 minutes.
5) Tip the flask to allow the monolayer to slide down the surface.
6) Add 3 ml media (with serum) to the flask.
7) Resuspend the cells in the media by repeatedly gently pipetting over the surface of the flask.
8) Add 9 ml of fresh media with serum to 3 new 25 cm^2 flasks (properly labeled).
9) Add 1 ml of cell suspension to each of the 3 new flasks.
10) Cap new flasks and return to incubator.

Notes:

- This procedure assumes use of 25 cm^2 tissue culture flasks.
- The total amount of media in a flask should be approximately 0.2–0.5 ml / cm^2. More than this amount will limit gaseous diffusion through the media to the cells.

Type	Size	Area (cm^2)	Minimum (ml)	Maximum (ml)	Standard (ml)
Flask	T-25	25	5 ml	12.5 ml	10
	T-75	75	15 ml	37.5 ml	25
	T-150	150	30 ml	75 ml	50
Petri Dish	60 mm	21	4.2	10.5	8
	100 mm	55	11	27.5	20
Plate	6-well	9.5	1.9	4.75	3.5
	12-well	3.8	0.76	1.9	1.5
	24-well	1.9	0.38	0.95	0.75
	48-well	0.8	0.16	0.4	0.3
	96-well	0.32	0.064	0.16	0.15

- The activity of trypsin is negated in the presence of serum. Thus each flask should be washed with a serum free media prior to trypsinization.
- 1mM EDTA can be substituted for trypsin if the adherent cells are difficult to remove. When using EDTA, either:
    - substitute EDTA for trypsin and follow step 3 in the above protocol exactly, or …
    - prewash flask with 3-5ml EDTA, remove, then trypsinize as in step 3 above, or …
    - use EDTA only (5ml/25cm^2 flask) and leave on for 5-15 minutes, then resume at step 5.
- It should be noted that EDTA may be toxic to some cell types.