

Sub-culturing attached cell lines requiring trypsin

(adapted from Cell culture guidelines- Abcam)

Note: not all cells will require trypsinization, and to some cells it can be toxic. It can also induce temporary internalization of some membrane proteins, which should be taken into consideration when planning experiments. Other methods such as gentle cell scraping, or using very mild detergent can often be used as a substitute in these circumstances.

1. When ready, carefully pour off media from flask of the required cells into waste pot (containing approximately 100 ml 10% sodium hypochlorite) taking care not to increase contamination risk with any drip;
2. Using aseptic technique, pour/pipette enough sterile PBS (in T180 use 20 mL; in T75 use 10mL; in T25 use 5mL) into the flask to give cells a wash and get rid of any FBS in the residual culture media. Tip flask gently a few times to rinse the cells and carefully pour/pipette the PBS back out into waste pot. This may be repeated another one or two times if necessary (some cell lines take a long time to trypsinize and these will need more washes to get rid of any residual FBS to help trypsinization);
3. Using pipette, add enough trypsin EDTA solution (0,25%) to cover the cells at the bottom of the flask. e.g. in T25 flask approx 1 ml; in T75 flask approx 3-5 ml; in T180 flask approx 10 ml;
4. Roll flask gently to ensure trypsin contact with all cells. Place flask in 37°C incubator. Different cell lines require different trypsinization times. To avoid over-trypsinization which can severely damage the cells, it is essential to check them every few minutes.
5. As soon as cells have detached (the flask may require a few gentle taps) add some culture media to the flask (the FBS in this will inactivate the trypsin).
6. Using this cell suspension, pipette required volume of cells into new flasks at required split ratio. These flasks should then be topped up with culture media to required volume: e.g. in T25 flask approx 5-10 ml; T75 cm2 flask approx 10-30 ml; T180 cm2 approx 40-150 ml;

Leave cells overnight to recover and settle. Change media to get rid of any residual trypsin.

Changing media

1. If cells have been growing well for a few days but are not yet confluent (e.g. if they have been split 1:10) then they will require media changing to replenish nutrients and keep correct pH. If there are a lot of cells in suspension (attached cell lines) or the media is starting to go orange rather than pinky orange then media change them as soon as possible.

2. To media change, warm up fresh culture media at 37°C in water bath or incubator for at least 30 mins. Carefully pour of the media from the flask into a waste pot containing some disinfectant. Immediately replace the media with 100 ml of fresh pre-warmed culture media and return to CO₂ 37°C incubator.