



Passaging/Splitting Cells

Before you start

See Medium & Reagents for JM8.F6 cells' protocol for all reagents
Thaw and pre-warm trypsin
Pre-warm PBS
Pre-warm required media
Pre-warm gelatin

Dish/Flask/Plate	Volume of Gelatin/PBS	Volume of Trypsin	Volume of Media
6 well plate	3ml	500µl	4.5ml
96 well plate	100µl	25µl	175µl
10cm Petri dish	8ml	1.5ml	8.5ml

Things you'll need

An aspirator & sterile filter-free tips/pipettes
Pipette aid & plastic pipettes
3 x liquid reservoirs
Multichannel pipette and 200µl Rainin filtered tips.
We have tried several brands of tips, and find that the bore of the Rainin 200µl tip is optimal for dissociating cells.

For passaging cells in 96-well plates (Falcon #353916) **or 10cm² Petri dishes** (Corning #430167):

Example: Passing a confluent 10cm Petri dish into 4 daughter Petri dishes.

1. Remove the parent 10cm Petri dish from the incubator and observe under the microscope. Check cells are healthy and confluent
2. Aspirate old media
3. Wash with 10 ml pre-warmed PBS
Add PBS to the side of the dish, and slowly tilt dish to gently wash the cells.
4. Add 1.5 ml pre-warmed trypsin.
Gently swirl the dish to cover all cells with trypsin
5. Incubate at 37° for 15 minutes
6. While cells are incubating, remove medium from fresh feeder plates and add fresh, pre-warmed medium
7. After incubation, gently swirl the plate again and add 8.5 ml medium to inactivate the trypsin.
Pipette up and down gently 3 to 4 times to disperse cells
8. Transfer 2.5 ml of the cell suspension to each of the four fresh feeder plates.
Swirl the plate to distribute the cells evenly across the plate
9. Incubate the plates in an incubator at 37°C with 5% CO₂.
10. Check cells the following day and media change to remove all traces of trypsin and dead cells.



Example: Passing a confluent 96 well plate into 4 daughter plates.

1. Coat four fresh, sterile 96-well tissue culture plates with 100 μ l gelatin.
Incubate at room temperature for >10 minutes.
2. Remove the parent 96-well plate from the incubator and observe under the microscope. Check cells are healthy and confluent
3. Aspirate old media
4. Wash each well with 100 μ l pre-warmed PBS
Add PBS to the side of the wells
5. Add 25 μ l pre-warmed trypsin.
Gently tap the plate to ensure all cells are covered with trypsin
6. Incubate at 37^o incubator for 15 minutes
7. While cells are incubating, remove gelatin from the daughter 96-well plates and add 150 μ l fresh, pre-warmed medium to each well
8. After incubation, gently tap the plate again and add 175 μ l medium to inactivate the trypsin.
Gently pipette up and down 3 to 4 times to disperse cells.
9. Gently transfer 50 μ l of the cell suspension to each of the four daughter 96-well plates.
10. Incubate the plates in an incubator at 37°C with 5% CO₂.
11. Check cells the following day and media change to remove all traces of trypsin and dead cells



12. For splitting 96-well plates:

Example: Splitting a 96-well plate 1:4.

- 1) Gelatinise 4 X 96-well plates with 100µl 0.1% gelatin.
- 2) Remove the 96-well plate from the incubator and observe under the microscope. Check cells are healthy and confluent.
- 3) Using multichannel aspirator, remove old media from wells.
- 4) Using electronic multichannel pipette, add 100µl of PBS to each well.
- 5) Aspirate off PBS.
- 6) Add 25µl 2 x Trypsin + glucose.
- 7) Gently tap plate and incubate for 8-10 minutes.
- 8) While cells are incubating, remove gelatin using aspirator and add 150µl of pre-warmed media to each well.
- 9) After incubation, gently tap the plate again, and check that cells have lifted off the plate.
- 10) Add 175µl of media to the cells.
- 11) Use the Eppendorf multichannel pipette and the 200µl Rainin tips for the split:
 - i. Use a fresh tip for each well i.e. a whole box of 96 tips will be required
 - ii. Gently aspirate the first column of the master plate, up and down about 5 times, then transfer 50µl of cell suspension to the first column of each of the new plates, gently mixing the cell suspension in the new media.
 - iii. Discard tips, and repeat for the next 11 columns.
- 12) Label plates and incubate.
- 13) Check cells the following day and media change to remove all traces of trypsin and dead cells.