Passaging/Splitting Guide

Plate	Gelatin/PBS	Trypsin	Media (add to inactivate trypsin)	Media (daily feeding)
96-well	100ul	25ul	175ul	200ul
24-well	400ul	100ul	700ul	800ul
6-well	2ml	500ul	2.5ml	2-3ml
10cm plate	5ml	2ml	8ml	10ml

E14 Medium

Glasgow Minimum Essential Medium	Sigma	G5154	404.5ml
FBS (single lot tested)	Hyclone	SV30014	75ml
Glutamin-I, 100X	Gibco	35050-061	5ml
Non-essential amino acids, 100X			5ml
Pen-Strep, 100X			5ml
Sodium pyruvate, 100X			5ml
2 (beta)mercaptoethanol *fresh aliquots (1000X stock = 70ul in 10ml PBS w/o Ca or Mg)	Sigma	M-7522	500ul
LIF (Esgro), 10^7U/ml	Gibco	13275-029	50ul

Methods:

1. Prepare gelatin-coated plates by incubating plates with 0.1% gelatin in a 37Ã‹Å¡C incubator for 1~2 hours.
2. Change media 2-4 hours prior to splitting. Cells must be 60-80% confluent and healthy.
3. When cells are ready to be passaged, aspirate off old media and wash with 10ml pre-warmed PBS. Add PBS gently to the side of the plate to avoid disrupting the cells.

4. After aspirating PBS, add 2ml of pre-warmed trypsin. Be sure to cover the entire plate by carefully swirling it.

5. Incubate in a 37C incubator for 5 minutes and check under the microscope. If you see big clumps of cells, incubate additional 2-5 minutes. Do not over-trypsinize.

6. While cells are incubating, remove gelatin from the pre-treated plates and add 5ml of warmed medium.

7. Once majority of the cells are uniformly dispersed into small clumps or single cells, inactivate trypsin by adding 8.5ml of medium. Gently pipette up and down using 10ml pipette 10-15 times without making bubbles.

8. Transfer cell suspension to new plates, so the final confluence is between 20-30%, depending on your needs. Do not split below 20% confluency.