Mouse Splenocyte Separation

General Surgery Lab SOP

7-24-2013

Complete media (good for 2 weeks)

- RPMI 1640 500mL
- L-glutamine 5mL
- Penicillin-streptomycin 5mL
- Sodium pyruvate 5mL
- Non-essential amino acids (NEAA) 5mL
- 2-mercaptoethanol (2-ME) 0.5mL
- Sterile filter and store at 4ºC

10% FBS-Complete media

- Fetal Bovine Serum (heat activated) 10mL
- Complete media 90mL
- Sterile filter and store at 4ºC
- Make fresh daily

Collagenase D suspension (1mg/mL in 10% FBS-RPMI ó can use the 10% FBS-complete media)

- Weigh out Collagenase D in mg
- Mix with equal parts (in mL) of 10% FBS-RPMI
- Sterile filter
- Store in 5mL aliquots at -20ºC

RBC (1X) Lysing Buffer (can be purchased)

- Ammonium chloride (NH_4Cl) 8.26g
- Potassium bicarbonate ($KHCO_3$) 1g
- EDTA 0.037g
- ddH_2O 1L
- Mix well
- pH to 7.4
- Sterile filter
- Store up to 6 months at room temp or 4ºC

Splenocyte Separation

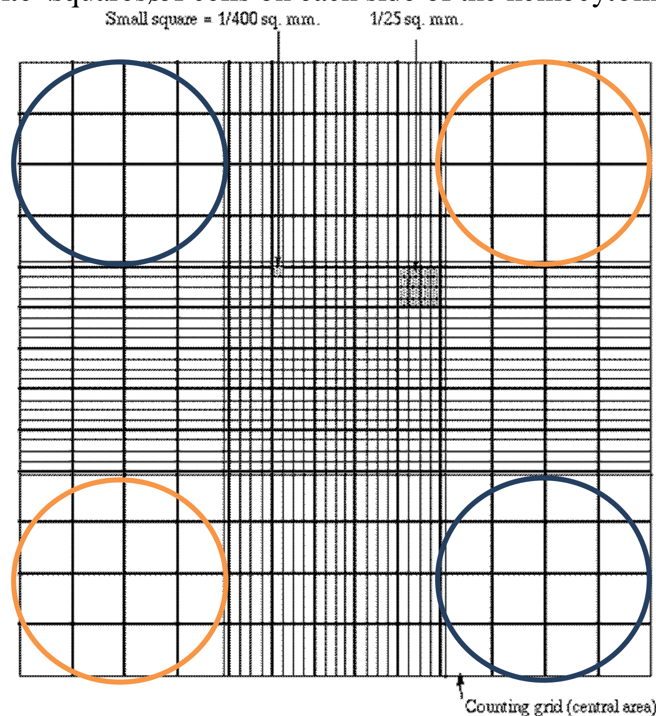
1. Harvest mouse spleen and collect in 1.5mL eppendorf, with PBS (1mL) on ice.
    a. If splenocyte suspension is to be used for culture, all the following manipulations should be performed under the hood in a sterile fashion.
2. Inject 1ml (tuberculin syringe) of room temp collagenase D into the spleen and then cut the spleen into pieces with scissors as finely as possible and leave in petri dish (in the leftover 4ml collagenase D).
3. Incubate spleen for 30mins -1hour at 37ºC.
4. Use the end of the barrel of a 10ml syringe to smash the spleen pieces within the petri dish and then to further break up the last remnants use a 10ml pipette and draw the

solution up and down about 10 times ó each time closing the end of the pipette against the bottom of the petri dish ó to forcefully expel the contents and break up the pieces in doing so.

5. Pipette the solution through a sterile 40 or 70 m mesh strainer into a 50ml conical tube. Use PBS to wash out the petri dish, and pour this through the mesh strainer as well. Fill the conical tube up to approx 45ml.
6. Centrifuge the 50ml tube for 10mins at 250G or 1200rpm at 4ºC.
7. Discard the supernatant. Thoroughly re-suspend the pellet in 3ml RBC(1X) lysing buffer at room temp for 3mins, and shake the tube vigorously every 1 min. Then pour PBS into the 50ml tube again to the 45ml mark.
8. Centrifuge the 50ml tube for 10mins at 250G or 1200rpm at 4ºC.
9. Decant. Re-suspend pellet in 10ml 10%FBS-Complete media. Leave on ice.
10. Count cells.

Count cells with hemocytometer

1. Add 190 L Trypan blue to 10 L of the above cell suspension into an 1.5mL eppendorf tube (1:20 dilution)
    a. Thoroughly vortex the suspension before removal of cells and also after addition to the trypan blue
2. Put 10 L of this suspension into each side of the hemocytometer.
3. Count 2 opposite ÷squaresø of cells on each side of the hemocytometer.



4. Average your counts together and multiply by 10 to obtain your cell count.