Preparation of Tissues for Cell Culture

This process can be used for lymph nodes spleens, thymii and various other tissues.

1. Remove whole spleen from mouse and place in RPMI in aseptic conditions.
2. Macerate the tissue using the plunger end of a 3cc syringe until completely dissociated.
3. Pass the tissue/cell suspension through a 70um cell strainer (BD Biosciences, San Diego, CA) to remove excess tissue/particulate.
4. Centrifuge at 1000rpm for 10 minutes and resuspend in 1mL of red blood cell lysing buffer (Sigma Aldrich, St. Louis, MO) to lyse red blood cells.
5. After 1 minute, add the cell suspension to RPMI and wash a total of 3 times.
6. After the third wash step, take a sample of cells (20μL) and count using a hemacytometer and equal volume Trypan Blue (Gibco, Carlsbad, CA) as an exclusion dye.
7. Resuspend the cells in cRPMI (complete media) supplemented with FBS (10%), Penicillin /Streptomycin (Gibco Cat.# 10378-016, 100U/mL), L-glutamine (Gibco, 292μg/mL), non-essential amino acids (Gibco Cat.# 11140-050), sodium pyruvate (Gibco Cat.# 11360-070, 1mM) and 2-mercaptoethanol (2ME, Gibco, 0.00034%).