Title: **Collection and Cryopreservation of Lymphocytes from Lymph Node or Splenocytes from Spleen**

1.0 Procedure

1.1 Tissue Processing:

1.1.1 Using the forceps, remove the tissue from the container of tissue transport media (TTM) and place in an appropriately labeled petri dish.

1.1.2 Carefully mince the tissue into 3-4 mm size pieces with a sterile scalpel and transfer the pieces to the 50 mL conical labeled “unfiltered”.

1.1.3 Using a serological pipet, rinse the petri dish with 5 mL of TTM and transfer the rinse to the 50 mL conical containing the tissue. Repeat.

1.1.4 Pipet the tissue and media mixture up and down multiple times to further break up the sample, releasing the cells.

1.1.5 Using the pipet, pull up the supernatant, leaving behind as much of the larger pieces of tissue as possible and pipet the media through the 70 µm filter into the tube labeled “70 µm”.

1.1.6 Rinse the minced tissue in the “unfiltered” tube with 10 mLs of TTM and pipet the rinse through the 70 µm filter, adding it to the tissue supernatant collected in the previous step.

1.1.7 Pipet the 70 µm filtered sample through the 40 µm cell filter into the tube labeled “40 µm”.

1.1.8 Centrifuge the tube labeled “40 µm”:

1.1.8.1 Temperature = Room Temperature

1.1.8.2 Speed = 300xg

1.1.8.3 Time = 10 minutes

1.1.8.4 Brake = ON

1.1.9 Pour off the supernatant into a waste container, being careful not to dislodge pellet.

1.1.10 Mechanically disrupt the pellet by flicking the tube with your fingers or other means. Do not vortex.

1.1.11 Resuspend the sample in 5 – 10 mLs of DPBS and count the cells.

1.1.12 Centrifuge the cells

1.1.12.1 Temperature = Room Temperature

1.1.12.2 Speed = 300xg

1.1.12.3 Time = 10 minutes

1.1.12.4 Brake = ON

1.1.13 Pour off the supernatant into the liquid waste container. Carefully removing as much as possible, without disturbing the pellet, by decanting and tapping the conical tube onto a dry paper towel to remove any excess liquid.

1.1.14 Mechanically disrupt the pellet by flicking the tube with your fingers or other means. Do not vortex.

1.1.15 Slowly add freezing media (90%FBS, 10%DMSO) drop-wise to the cells while simultaneously swirling the tube.

1.1.16 Transfer aliquots to a CoolCell.

1.1.17 Place the CoolCell in a low-temperature freezer (-70 - -80˚C) overnight.

1.1.18 Aliquots must be transferred to a -150°C or LN2 freezer for long-term storage within 2 weeks from the date placed in -80°C, optimally the transfer should take place the next working day.