# Bullet Blender™ Protocol
# Extraction of Lymphocytes from Heart

The protocol described in this document is for the use of the Bullet Blender™ for the extraction of viable lymphocytes from spleen.  This protocol was developed using human cadiac tissue; note that the time and speed settings may differ somewhat due to the variation in size and toughness of tissue from species to species.  Some buffers and reagents have been specified, but you may substitute these for other analogous buffers / reagents if desired.

**Materials Required:**     heart , Bullet Blender™, scalpel, fetal bovine serum, collagenase IV, Dnase, phosphate-buffered saline, 40 micron cell strainer, Lymphocyte M, pipettor, microcentrifuge tubes, and 3.2mm stainless steel beads (part # SSB32)

## Instructions

1. Cut cardiac tissue into a few pieces and place into a microcentrifuge tube containing 1ml heart digest medium (10% fetal bovine serum, 1 mg/ml collagenase IV, and 0.02 mg/ml Dnase in PBS).  Do not use more than 300mg of cardiac tissue per tube.
2. Add two 3.2mm stainless steel beads to each tube.
3. Incubate at 37°C for 20 minutes.
4. Place the microcentrifuge tubes into the Bullet Blender™.
5. Set controls for **SPEED 2** and **TIME 3** minutes.  Press **Start**.
6. Re-incubate the tubes at 37*C for 20 minutes.
7. Using a 40 micron cell strainer, crush the tissue and transfer to fresh tubes.
8. Resuspend cardiac cells in 5ml PBS
9. Overlay 5 ml of Lympocyte M (Cedarlane) with 5 ml of the cardiac cell suspension.
10. Spin at 1500 g for 20 min (at room temperature)
11. Collect the lymphocytes at the interface.  Wash cells once in cold PBS.
12. Proceed with your downstream application.

## SAFETY NOTE!!!
**When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.**

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