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Bullet Blender™ Protocol
Extraction of Splenocytes from Spleen

The protocol described in this document is for the use of the Bullet Blender™ for the extraction of viable splenocytes from spleen. This protocol was developed using murine spleen; 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: spleen, Bullet Blender™, scalpel, ACK Lysis buffer, pipettor, microcentrifuge tubes, and 3.2mm stainless steel beads (part # SSB32)

Instructions

1. Cut spleen into a few pieces and place into a microcentrifuge tube containing 1mL of ice-cold PBS. (no more than 300mg of spleen per tube)
2. Add two 3.2mm stainless steel beads to each tube.
3. Close the microcentrifuge tubes and place them into the Bullet Blender™.
4. Set controls for **SPEED 2** and **TIME 3** minutes. Press **Start**.
5. After the run, remove tubes from the instrument. Visually inspect samples. Samples should appear cloudy with no visible chunks of tissue.
6. If chunks of tissue are still present, homogenization is unsatisfactory, run for another two minutes at speed 3**.**
7. Lyse Red Blood Cells using ACK Lysis buffer (BioWhittaker) or other appropriate method
8. 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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