Splenocyte Preperation

Rabia Khan

Abstract

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Protocol

Step 1.
Harvest mouse spleen and prepare a single cell suspension. Use slides or a syrings to push the spleen through a cell strainer.

Step 2.
Pellet the cells by centrifugation (350 x g); aspirate the supernatant.

Step 3.
Dilute the 10X Red Blood Cell Lysis Buffer to 1X working concentration with deionized water and resuspend the pellet in 5 ml of 1X Lysis Buffer.

Step 4.
Incubate on ice for 4-5 minutes with occasional shaking.

Step 5.
Stop the reaction by diluting the Lysis Buffer with 20-30 ml of 1X PBS.

Step 6.
Spin the cells (350 x g) and discard the supernatant.

Step 7.
Resuspend the pellet in the appropriate buffer

Step 8.
Count cells, adjust density, and proceed with cell staining procedures.