



University of New South Wales
School of Medical Sciences

LABORATORY HANDOUT

ANAT3231 Cell Biology 2010

Lab 11 : Stem Cell Isolation from Mouse Spleen

Lab 12 : Stem Cell Analysis

Dr Antonio S Lee

ANAT3231 LAB 11 / 2:00 - 4:00 pm / 26 May 2010

6 groups (5 students per group)

Personal Protective Equipment (PPE) provided:

Laboratory gown

Gloves

Materials Provided for Communal Use:

Esky containing crushed ice / group

Timer / group

Pipettes: 20uL, 100uL and 1mL (2 - 3 sets will be available)

Blue and yellow pipette tips

Biological Waste Container

(Access to Centrifuge 300G) – *this step will be performed by a staff member*

Transfer pipettes

Haemocytometer

Trypan Blue

15mL Falcon tubes (polypropylene) + tube racks

Magnetic Cell Sorting magnets & stand

Introduction

2:00 - 2:05 Introduction by Dr Antonio Lee

2:05 - 2:30 Introduction on MACS (Magnetic Activated Cell Sorting) Technology by Ms Vittoria Lazzazro (Miltenyi Biotec)

Experimental Protocol

2:30pm - 3:00pm gentleMACS (gMACS)

- 6 spleens from 6 mice are kept on ice
- each group will receive 1 x spleen and 1 x gMACS tube (purple capped)
- 2 groups will perform cell suspension by processing x 2 tubes at once
- follow gMACS program for 'mouse spleen'
- *please refer to 'Preparation of single-cell suspensions from mouse spleen' protocol*

- cells from all 6 groups will be pooled
- total cells with Trypan Blue are counted using haemocytometer
- cells are divided into 8 x 15mL Falcon tubes (1×10^7 total cells/tube)
- each group will receive 1 x Tube on ice

3:00pm - 4:00pm MACS Technology

- each group will perform CD4 protocol (cell labeling and separation)
- *please refer to 'CD4 (L3T4) MicroBeads' & 'FcR Blocking Reagent' protocol*
- prior to cell separation 100uL should be taken for flow-cytometry (original sample)
- following separation, each group is to hand-in 'positive' and 'negative' fractions

ANAT3231 LAB 12 / 2:00 - 4:00 pm / 2 June 2010

There is No Wet-Lab Component in Today's Lab

2:00 - 2:45 Introduction to Flow-Cytometry and Fluorescence Activated Cell Sorting (FACS) by Dr Antonio Lee

2:45 - 3:30 Analysis of Flow-Cytometry data on the separated cell populations from Lab 11.

3:30 - 4:00 Discussions & Time for the Group Projects

Note: *Please refer to 'CD4 antibodies' information & protocol.*