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Laser Microdissection of Bone Marrow Cells for Molecular Analysis

PROTOCOL #8

Bone Marrow Application

Bone marrow is the soft tissue that fills the cavities of bone and is the site of hematopoiesis. It is the source of pre-cursors to all blood cells: red blood cells, white blood cells and platelets. Since there is a constant turnover of these cell types in the blood, the bone marrow cells are in various developmental stages to replace cells lost in the blood stream. Study of different types of bone marrow cells will help in our understanding of the molecular mechanisms underlying hematopoiesis. This protocol describes how specific bone marrow cells can be microdissected from biopsies of bone marrow, allowing for RNA from these cells to be extracted for molecular analysis.

Tissue Preparation

A human bone marrow biopsy was harvested using standard techniques. The various components contained within the biopsy, such as bone marrow cells, bone chips and red blood cells, were separated by centrifugation. Only the layer containing the bone marrow cells was fixed in 10% neutral buffered formalin and processed into paraffin blocks. Using a standard rotary microtome, sections were cut and mounted onto polyethylene napthalate (PEN) membrane slides. The sections were stained with Cresyl Violet to identify plasma cells within the sample following the procedure detailed below.

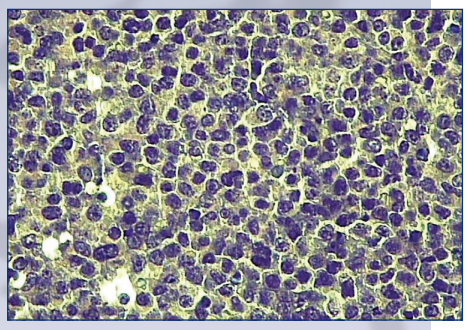
Preparation of 1% Cresyl Violet Stain

Material	Quantity
Cresyl Violet	1.0g
Water, nuclease-free	100ml
Allow staining solution to sit for 24 hours at room temperature before use. Filter solution before use.	

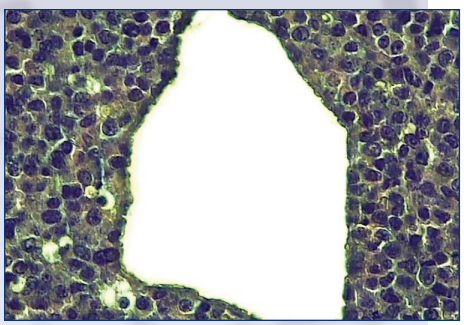
Cresyl Violet Staining Procedure

Step	Process	Time
1.	Xylene	2 minutes
2.	Xylene	2 minutes
3.	100% ethanol	1 minute
4.	95% ethanol	1 minute
5.	75% ethanol	1 minute
6.	Water, nuclease-free	30 seconds
7.	1% Cresyl Violet	30 seconds
8.	75% ethanol	30 seconds
9.	95% ethanol	30 seconds
10.	100% ethanol	30 seconds
11.	Xylene	5 minutes
12.	Air dry in fume hood	5 minutes minimum
13.	Proceed immediately to microdissection	

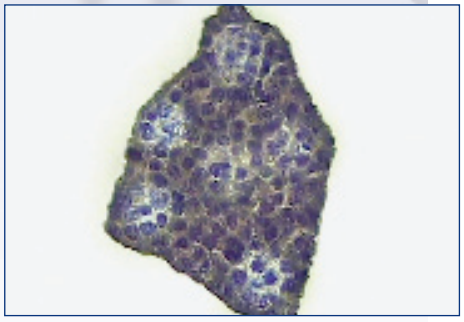
Figure 1. Microdissection of bone marrow with the Veritas™ Microdissection System:



A: Before microdissection.



B: Tissue section after laser microdissection.



C: Microdissected bone marrow cells on the CapSure Macro LCM Cap.

Laser Microdissection

Standard laser cutting and laser capture microdissection procedures were used to collect areas of interest from the tissue section following the procedure as listed below.

Microdissection Procedure

Step	Procedure
1.	Set laser cutting power to lowest possible setting that will still allow full cutting through the PEN membrane
2.	Scan slide for area(s) of interest. While scanning, store the location(s) of interest to be retrieved later for collection by using "Stored Position" feature in the "Microscope" window.
3.	Place a CapSure® Macro LCM Cap onto a field containing desired area(s) for collection.
4.	Outline area(s) of interest that will be cut from tissue section. In the Microdissection window under the tab identified as "Cut and Capture", use drawing icon to draw around the area(s) that will be collected. Click on the icon for "Go Capture" to activate, this will initiate laser cutting and automatically provide the laser capture "tack points" to attach your sample to the LCM cap.
5.	When the desired cells have been collected, remove the CapSure Macro LCM Cap containing these cells.

RNA Extraction

RNA is extracted from the microdissected bone marrow cells using the Paradise™ Reagent System. Isolated RNA can now be used for downstream molecular analysis.

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