Creighton UNIVERSITY

# Cell Separation using Lympholyte-M

(Cedarlane Laboratories)

**Equipment:**

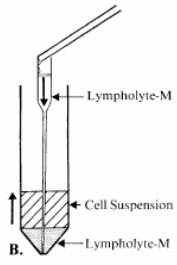
15 ml Conical tubes
Pasteur pipettes

**Reagents:**

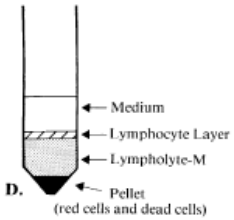
HBSS or DPBS
Cell Preparation
Lympholyte-M (Cedarlane #CL5031)
RF10 (or RF10-M) media

**Method:**

1. Shake Lympholyte-M well.
2. Warm cell suspension and Lympholyte-M to **room temperature**.
   - Keeping reagents at room temperature is very important in this protocol, as the density of Lympholyte-M is temperature dependent.
3. Resuspend cell preparation in HBSS or DPBS to a maximum of $2x10^7$ cells/ml.
   - *Note: If cell suspension contains a large amount of debris or erythrocytes, a cleaner separation will be obtained if the cell concentration is set at $1x10^7$ cells/ml.*
4. Underlayer an equal volume of Lympholyte-M (up to 5ml) using a Pasteur pipette.
   - See figure "B" below.
5. Centrifuge 20 minutes at 1250g at room temperature.
   - *Note: My original instructions said to centrifuge at 500g for 20 minutes.*
6. Remove viable lymphocytes from the Lympholyte/media interface.
   - See figure "D" below.
   - Discard excess media, Lympholyte-M and pellet.
7. Dilute the isolated cells with RF10-M and centrifuge 10 minutes @ 800g.
8. Discard supernatant and wash the cells 2-3 times RF10-M before further processing.




**Underlayering Lympholyte-M**




**After Centrifugation**

**Notes:**

- *When separating spleen cells, the interface will contain macrophages, while granulocytes and plasma cells pellet.*
- *When separating bone marrow cells, the interface will be significantly contaminated with granulocytes.*
- *Use Lympholyte-Mammal to separate out lymphocytes from peripheral blood.*