



Eosinophil Isolation Kit (Enough for 25 Samples)

CATALOG NUMBER: IMM002

LOT NUMBER:

STORAGE/HANDLING: Maintain kit materials at 2-8°C up to the expiration date.

MATERIALS PROVIDED:

1. Osmotic Lysis Buffer (10X) - (Part No. 90574) One bottle containing 80 mL.
2. Neutralization Buffer (10X) - (Part No. 90575) One bottle containing 80 mL.
3. Eosinophil Counting Stain - (Part No. 90576) One tube containing 2 mL.
4. Resuspension Buffer - (Part No. 90577) One bottle containing 20 mL.

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Precision pipettes
- 15 mL conical tubes
- Eppendorf tubes
- Sterile PBS or appropriate medium
- Filcon™ disposable filter device (optional)
- Tabletop centrifuge
- Bright field microscope
- Hemocytometer or other means of counting cells
- Trypan blue or equivalent viability stain (optional)

REAGENT PREPARATION:

- Osmotic Lysis Buffer (10X) - Shake well before each use ensuring that the stock solution is completely mixed. Dilute 1:10 in deionized water and mix thoroughly.
- **All other reagents are provided ready to use.**

SPECIMEN REQUIREMENTS:

- EDTA-anticoagulated peripheral blood with hypereosinophilia (>800 eosinophils/ μ L). **Note:** citrate or heparin anticoagulated blood samples are **not** suitable for this kit.
- Blood samples should be less than 6 hours old for best results; older blood samples will have decreased yields; blood stored longer than 24 hours should not be used.

POSSIBLE SPECIMEN PROBLEMS:

- Low initial eosinophil count
- Old blood (24 hours or greater)

BACKGROUND:

Eosinophils are terminally differentiated leukocytes that protect the body from allergic diseases, parasitic infections, and other disorders. Typically residing in submucosal tissue, eosinophils are recruited to the infection site where they discharge their toxic, granular contents to fight infection.

In normal blood, eosinophil values are typically 0-400 cells/ μ L and account for 0-3% of WBC count. However, during eosinophilic leukocytosis, eosinophils increase to >1000 cells/ μ L and 5-90% of the WBC count. Increased eosinophil counts (eosinophilia) are most often associated with allergic reactions and parasitic infestations, but can also result from other disorders (e.g. skin diseases, autoimmune diseases, asthma, hay fever, Hodgkin's disease, leukemia).

Chemicon's Eosinophil Isolation Kit provides a quick, efficient method for purifying eosinophils from EDTA-anticoagulated blood samples. After purification, eosinophils can be stained/counted in a hemocytometer or further assayed. To confirm purification results, the kit includes an Eosinophil Counting Stain that selectively stains eosinophils (other WBC's are lysed).

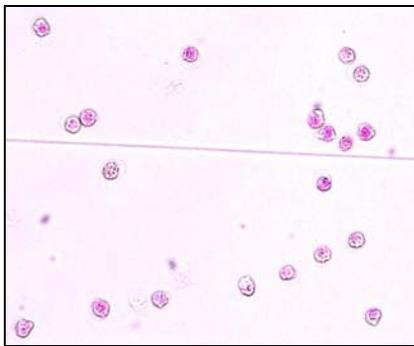
EOSINOPHIL ISOLATION PROCEDURE (TO BE PERFORMED AT ROOM TEMPERATURE):

1. Mix EDTA-anticoagulated blood specimen well with gentle agitation or tube inversion prior to processing.
2. Add 0.5 mL of EDTA-blood to a 15 mL conical tube.
3. Add 8.5 mL of 1X Osmotic Lysis Buffer to the blood. Cap tightly and gently invert the tube several times for 60 seconds.
4. Neutralize the sample by adding 1 mL of 10X Neutralization Buffer.
5. Centrifuge the sample for 5 minutes at 1200 rpm (equivalent to 300 x g) in a tabletop centrifuge.
6. Carefully aspirate the supernatant, leaving a red-brown pellet in the tube.
7. Resuspend the pellet in 9 mL of 1X Osmotic Lysis Buffer (titurate to generate a single cell suspension). Cap tightly and gently invert the tube several times for 60 seconds.
8. Neutralize the sample by adding 1 mL of 10X Neutralization Buffer.
9. Centrifuge the sample for 10 minutes at 1200 rpm (equivalent to 300 x g) in a tabletop centrifuge.
10. Carefully aspirate the supernatant, leaving a tan pellet in the tube.
Note: At this point, the enriched cell pellet should comprise >70% pure eosinophils. If erythrocyte layer is visible, repeat steps 7-10. If additional purification is desired (to achieve >80% purity), repeat steps 7-10.
11. Resuspend the eosinophil pellet in 250 μ L of Resuspension Buffer or other desired medium (titurate to generate a single cell suspension).
Note: If cell debris or fibrin fibers (threads) are visible, the cell suspension may be filtered (Falcon™ or equivalent) to remove them.
12. Cell suspension will remain viable for at least 2 hours at 2-8°C.

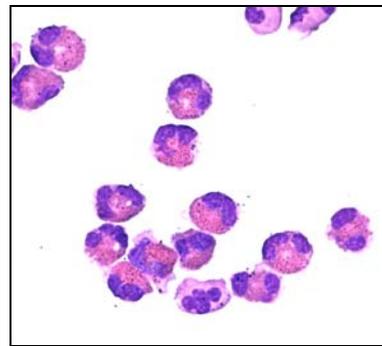
EOSINOPHIL STAINING PROCEDURE:

1. Mix 50 μ L of Eosinophil Counting Stain with 50 μ L of isolated eosinophil suspension (step 12 above).
2. Transfer the mixture to a hemocytometer/counting chamber.
3. Allow the mixture to stand for 15-30 minutes.
4. Visualize eosinophils under a microscope, appearing bright pink.
Note: Counting Stain selectively stains eosinophils and lyses all other RBC's and WBC's.
5. Perform cell count.

Alternatively, a cytopreparation can be stained with Wright-Giemsa or Diff-Quik, following the manufacture's instructions, and examined microscopically.



Isolated eosinophils were stained with Eosinophil Counting Stain, then visualized by bright field microscopy (20X magnification).



Cytopreparation of isolated eosinophils were stained with Diff-Quik (**not included in the kit**), then visualized by bright field microscopy (60X magnification).

References:

1. Samoszuk M. (1997) *Histology and Histopathology*. **12**, 807-812.
2. Samoszuk, M., Deng, T., Hamamura, M., Su, M., Asbrock, N., and Nalcioglu, O. (2004) *The American Journal of Pathology*. **165**, 449-456.

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