Lumiprobe
Life science solutions

Reticulocyte Staining with Lumiprobe® Reticulocyte Stain

Reticulocytes are immature red blood cells formed in the bone marrow and released into the peripheral blood, where they mature into erythrocytes. An increase or decrease in reticulocyte count may indicate erythropoiesis activity or deficiency, particularly in disorders associated with anemia and bone marrow dysfunction.

In mammals, reticulocytes, like mature erythrocytes, lack a nucleus but retain residual organelles (ribosomes and mitochondria), as well as RNA and DNA. This characteristic distinguishes reticulocytes from mature erythrocytes. Nucleic acid stains, such as Lumiprobe® Reticulocyte Stain, reveal the mesh-like structure of ribosomal RNA (rRNA) in reticulocytes, allowing them to be visually differentiated from mature erythrocytes. Lumiprobe Reticulocyte Stain also enables the visual differentiation of the maturation stage of reticulocytes: new cells contain more RNA than mature reticulocytes, which have a lower RNA content.

Method Principle

Lumiprobe® Reticulocyte Stain is a ready-to-use solution for determining the number of reticulocytes in human peripheral blood. Thiazole orange binds to rRNA and DNA, forming a fluorescent complex with an absorption maximum at 509 nm and an emission maximum at 532 nm. Lumiprobe Reticulocyte Stain is suitable for both microscopy and flow cytometry applications.

Biomaterial

Blood should be collected in Vacutainer tubes containing EDTA. For optimal results, staining should be performed within 24 h of collection. Anticoagulated blood can be stored at room temperature (20...25 °C) for 24 h before staining.

Additional materials not supplied with the reagent:

- 1× Phosphate Buffered Saline (PBS)

Staining Procedure

1. Prepare two tubes: one for the control and one for the sample.
2. Add 2.5 µL of EDTA whole blood and 0.5 mL of PBS to the control tube and mix gently by vortexing.
3. Add 0.5 mL of Lumiprobe Reticulocyte Stain and 2.5 µL of EDTA whole blood to the sample tube and mix gently by vortexing.
4. Incubate the tubes for 30 min at room temperature in the dark.
5. The stained sample is ready for microscopy or flow cytometry.

Important! Measurements must be taken within 3 h after incubation. Samples must be stored in a dark environment. Immediately before measurement, the tube contents must be gently mixed by vortexing.

Important! When using a flow cytometer, the logarithmic scale for FSC and SSC must be set.

Note

- According to the literature, the percentage of reticulocytes in the blood is 0.9–2.1%. Newborns under two weeks of age have higher values [1].
- In some patients with very high reticulocyte counts, the threshold gate detected using an unstained (blank) tube may have a positive rate of more than 0.3%. In such cases, set the fluorescence threshold based on the base of the unstained peak.
- The Lumiprobe Reticulocyte Stain reagent should not be used with hemolyzed blood samples.
- Patients with macroplatelets (giant platelets) may produce abnormal results; thus, reticulocyte counts in these patients should be confirmed with an alternative method.
- Lumiprobe Reticulocyte Stain reagent is a nucleic acid stain. It labels DNA or RNA inclusions in red blood cells. Examples of such inclusions include nuclei of normoblasts (NRBCs), Jolly bodies (Howell-Jolly bodies), Cabot rings, malarial parasites, etc. If the reticulocyte count is excessively high, an additional reference method should be used, such as a Wright-Giemsa-stained peripheral blood smear.
- In patients with elevated white blood cell counts (as occurs in chronic lymphocytic leukemia), lymphocytes will be detected alongside red blood cells. In this case, the reticulocyte count should be confirmed by an alternative method.
- Patients receiving erythropoietin therapy may have an elevated reticulocyte percentage. Some patients may also have elevated numbers of NRBCs in the peripheral blood, which may interfere with reticulocyte assessment. The presence or absence of NRBCs can be confirmed by examining a Wright-Giemsa-stained peripheral blood smear.

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[1] Williams W, Beutler E, Erslev A, Lichtman M. Hematology. 4th ed. New York: McGraw-Hill; 1990.

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