

Reticulocyte Staining Solution

Product Code: TCL115

Product Information

All blood cells originate from bone marrow. The orthochromatic erythroblasts eject their cell nucleus and create reticulocytes which then enter the peripheral bloodstream. By removing endoplasmic reticulum the reticulocytes develop into a mature red blood cell within 4 days. The term reticulocyte originates from the web like structure which becomes visible after new methylene blue staining. Reticulocytes differ from other red blood cells in that they have a more convoluted shape and are about 8% larger than the mature RBCs. Reticulocytes contain some residual intracellular RNA.

Standard technique for staining reticulocytes is the new methylene blue staining. New methylene blue stains intracellular RNA blue in colour against pink stained RBCs. Depending on the stage of the reticulocyte the blue colour may be distributed in a general pattern (early reticulocytes) or punctate pattern (late reticulocyte). Cells with clear, blue cytoplasmic granules which can be seen without fine focusing are to be regarded as reticulocytes of maturation stage IV. From visual examination of a peripheral blood film so stained, the number of reticulocytes among 1000 reticulocytes is found and expressed as a percentage. This is termed the *reticulocyte count*.

TCL115 is a reticulocyte staining solution formulated to contain new methylene blue for staining reticulocyte in blood smear.

Composition

Reticulocyte staining solution is a proprietary formulation containing new methylene blue in biological buffer.

Significance of Reticulocyte Count

The reticulocyte count serves as an important tool to indicate shortened RBC survival and the subsequent appropriate response by bone marrow to increase the RBC production.

Normal Reticulocyte Count

Adults	0.5% - 2.5% of total RBC count
Infants	2% - 6% at birth. The count decreases to adult levels in 1 - 2 weeks

Abnormal Reticulocyte Counts

Reticulocyte count	Possible Clinical Conditions
Low	Iron deficiency anemia, folic acid deficiency, aplastic anemia, radiation therapy, liver cirrhosis, kidney disease, pernicious anemia
High	Acute bleeding, chronic blood loss, hemolytic anemia, kidney disease, erythroblastosis fetalis. The reticulocyte count may also increase after therapy for iron deficiency anemia or pernicious anemia.

Materials Required But Not Provided

Grease free microscopy slides (CG17)
Light microscope with 100X objective
Immersion oil
Clean test tubes
Micropipettes and tips
Dropper
Anticoagulated blood

Directions for Use

- In a clean and dry test tube mix blood and reticulocyte staining solution in 1:1 ratio.

Note: It is recommended to use freshly collected blood for reticulocyte staining, preferably within 3 hours of blood collection.

If precipitation occurs in the staining solution, filter it through Whatman filter paper. Precipitation and subsequent filtration will not affect performance and quality of staining.

- Incubate the mixture at 37°C for 15 - 20 minutes.
- Gently mix the solution again and create a thin film/ smear on clean grease free slide.
- Allow the film/ smear to dry.
- Observe the slides microscopically under 100X objective.
- Randomly pick the areas of the film where red blood cells are close to each other but do not touch or overlap.
- Count 1000 red blood cells including reticulocytes and determine percentage of reticulocytes using the formula-

$$\text{Percentage Reticulocytes} = \frac{\text{Total number of reticulocytes}}{1000} \times 100$$

- The value of percentage reticulocytes may be erroneous because of fluctuation in hematocrit (percentage volume of RBCs in blood) of a person. To account for variations caused by erythrocytes, the corrected reticulocyte count can be determined, as follows:

Gender (Adult)	Average normal Hematocrit (%)
Male	45
Female	42

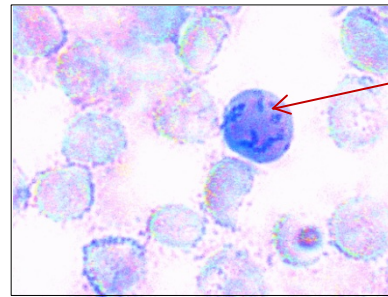
$$\text{Corrected Reticulocyte Count (\%)} = \frac{\text{Patient's hematocrit (\%)} \times \text{\% Reticulocyte Count}}{\text{Normal hematocrit (\%)} \text{ based on age and sex}}$$

- Reticulocyte counts are often corrected for anemia to get a more accurate reflection of the status of RBC production in patients. Absolute reticulocyte count gives the concentration of reticulocytes in patient's blood.

Absolute Reticulocyte Count = RBC Count X % Reticulocytes

The Reticulocyte Production Index (RPI) corrects the reticulocyte count for the degree of anemia.

$$\text{Reticulocyte Production Index (RPI)} = \frac{\text{Corrected Reticulocyte Count (\%)}}{\text{Expected Maturation Time (Days)}}$$



Reticulocyte with fragmented RNA stained with Reticulocyte Staining Solution

Blood smear stained with Reticulocyte staining solution (100X)

Quality Control:

Appearance:

Dark blue liquid

pH:

5.7 - 6.3

Osmolality in mOsm/kg H₂O:

600 - 640

Sterility:

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Storage and shelf life:

Store at 2 - 8°C away from bright light.

Shelf life is 12 months.

Use before expiry date given on the product label.

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Disclaimer:

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