



First for Pathology
A THERMO FISHER SCIENTIFIC COMPANY

Reticulocyte Fluid

For In-Vitro Diagnostics

For Professional Use only

Reticulocyte counting stain is used to detect the presence of reticulocytes by precipitating the residual ribosomal RNA within the reticulocytes to determine the number of reticulocytes present, expressed as a percentage of the total reticulocytes

Mode of Action

Nonnucleated immature erythrocytes contain nuclear remnants of RNA and the cell is known as a reticulocyte. To detect the presence of this RNA, the red cells must be stained while they are still living. This process is called supravital staining. With supravital staining, the RNA appears as a reticulum within the red cell. Using a supravital stain (e.g., new methylene blue), residual ribosomal RNA within the reticulocytes is precipitated. An equal volume of stain is added to EDTA-anticoagulated blood, the dilution mixture incubated, and a smear is prepared. The smear is examined to determine the number of reticulocytes present. An erythrocyte containing two or more particles of blue-stained material is a reticulocyte. The number of reticulocytes is expressed as a percentage of the total number of erythrocytes counted

Warning and Precautions

Reticulocyte Fluid is not classified as a hazardous substance.

Please consult the SDS and packaging labels before use.

Ingredients

Ingredients	CAS Number	Conc.
Sodium Chloride	7647-14-5	< 1%
tri-Sodium Citrate	64-04-2	< 1%
New Methylene Blue	61-73-4	< 10%
Water	7732-18-5	Balance

Stability

Reticulocyte Fluid is stable for 12 months when stored at room temperature in a sealed container away from light and heat.

Sample Preparation

Fresh blood film or fresh EDTA anticoagulated blood film.

Technical Procedure

1. For venous blood, Add 3 or 4 drops of Reticulocyte fluid to an equal volume of well-mixed EDTA anti-coagulated blood in a small test tube and mix.

For peripheral (capillary) blood, draw blood up in a leukocyte diluting pipette, then draw in an equal volume of Reticulocyte fluid. Alternatively, mix approximately equal volumes of blood and stain solution on a clean slide and draw up the mixture into a capillary pipette.

2. Equal volumes of blood and stain give optimum staining conditions. An excess of blood causes the reticulum to under-stain. An excess of stain usually obscures the reticulum

3. Draw the blood and stain up into the bulb of the pipette, mix well, and allow to stand for 10-15 minutes (10 minutes is usually sufficient) at room temperature (18-26°C). The staining time may be adjusted due to personal preferences for staining intensity. However, allowing the incubation time to exceed 15 minutes increases the possibility of erroneous results due to the dye adhering to mature erythrocytes

4. Mix again.

5. Expel a small drop of the blood-stain mixture onto a clean slide and smear in the usual manner. Make several slides.

6. Dry rapidly by waving slides in the air.

7. Coverslip and examine microscopically. If desired, the smears may be counterstained with Wrights Stain.

Technical Comments

Stained blood films are examined for the presence or absence of reticulocytes. A reticulocyte is any red blood cell containing two or more blue-stained particles. Using a low power (100x) oil immersion objective, pick an area of film where the cells are undistorted and the staining good. Count 1000 red blood cells including reticulocytes and calculate the proportion of reticulocytes as follows:

$$\text{Reticulocyte Count (\%)} = \frac{\text{Total Number of Reticulocytes}}{10}$$

Results and Interpretation

Only experienced and suitably qualified persons should carry out interpretation of stained slides.

Normal erythrocytes stain light greenish-blue. Reticulocytes stain deep blue and are sharply outlined



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Notes

This product is designed to be used at its full strength and does not require any further dilution or additive

Never leave the cap open.

Always prepare fresh solutions just before use and discard used solutions. Do not return used stains to its original container as this may adversely affect the overall performance of the unused product.

References

1. *Dacie, Sir John V & Lewis, S.M, Practical Haematology 11th Edition. Churchill Livingstone, New York USA, 2012*

2. *Clinical and Laboratory Standards Institute, Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry, and Supravital Dyes); Approved Guidelines – 2nd Edition; H44-A2, Vol 24 No 8*

Ordering Information

Code	Product Name	Size
FNNRETIC25	Reticulocyte Fluid	25 mL
FNNFG001	Reticulocyte Fluid	100 mL