

INTENDED USE

Reticulocyte Stain is intended for the identification of reticulocytes in blood films. Reticulocyte Stain reagents are for "In Vitro Diagnostic Use."

In 1949, Brecher¹ introduced the new methylene blue method for identification of reticulocytes based on precipitation of ribosomal RNA by the cationic dye. This has now replaced other methods and is the recognized procedure for the quantitation of reticulocytes in peripheral blood.²

Sigma-Aldrich provides a stable solution for the staining of reticulocytes in blood films. The blood and reticulocyte staining solution are mixed and incubated briefly at room temperature. Wedge or spun smears are made on microscope slides, air dried and evaluated under oil immersion on a light microscope. A red blood cell scoring positive would be observed containing two or more blue-stained granules.

REAGENT

RETICULOCYTE STAIN, Catalog No. R 4132

New methylene blue, 1% (w/v), sodium chloride, 0.9% (w/v), and potassium oxalate, 1.6% (w/v).

STORAGE AND STABILITY:

Store Reticulocyte Stain at room temperature (18–26°C). Reagent is stable until expiration date.

PREPARATION:

Reticulocyte Stain is supplied ready for use.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.

US and EU Risk and Safety Statements (Caution: Substances not yet fully tested)
Reticulocyte Stain reagent is an IRRITANT. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Blood may be collected in standard clinical laboratory evacuated tubes. All routine anticoagulants are acceptable (e.g., heparin, citrate and oxalate). The tripotassium salt of ethylenediamine tetraacetic acid (K₃ EDTA) is the anticoagulant of choice. If procedure is not carried out within 2–3 hours of collection, store blood at 4°C. Blood should be warmed to room temperature and mixed thoroughly prior to staining. Blood over 24-hours old is not recommended for use.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Microscope
Blood smearing instrument or Cytocentrifuge
Glass tubes, 10x75 or 12x75 mm
Laboratory droppers or Pasteur pipets
Microscope slides and coverslips

NOTES:

It is recommended that blood smears prepared from healthy donors be processed along with patient samples as normal controls.

A small amount of precipitate may form in the Reticulocyte Stain. If precipitate is noticed, filter through laboratory grade filter paper.

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE:

1. Add three drops of thoroughly mixed blood and two drops of Reticulocyte Stain, to a glass tube and mix well. Let stand 10 minutes at room temperature (18–26°C).
2. Prepare a conventional wedge or spun smear and air dry at least 15 minutes. Counterstaining is not recommended.
3. Coverslip and examine microscopically.

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PERFORMANCE CHARACTERISTICS

Stained blood films are evaluated subjectively for the presence or absence of reticulocytes. A reticulocyte is considered any red blood cell containing two or more blue-stained particles.²

Using a 100x oil immersion objective and a 10x ocular, randomly pick areas of the film where red cells are close to each other but do not touch or overlap. Count 1000 red blood cells including reticulocytes. The proportion of reticulocytes may be calculated as:

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

Normal adult values at sea level are $1.0 \pm 0.5\%$.²

Due to variability in the hematocrit, it may be necessary to correct the observed reticulocyte count to a normal hematocrit of 45%:

$$\text{Corrected Reticulocyte Count (\%)} = \frac{\text{Observed Count} \times \text{Measured Hematocrit (\%)}}{45\%}$$

In the normal non-anemic patient, reticulocytes are found in circulating blood on the fourth day of maturation following three days of maturation within the marrow. Factors increasing erythropoiesis may shorten bone marrow maturation time while lengthening blood maturation time. This shift results in large numbers of "shift" reticulocytes circulating in the blood and these should not be considered when quantitating reticulocytes as a reflection of red blood cell production.^{2,3} Shift cells may be detected by a Wright-stained film² and a reticulocyte production index² established on the corrected reticulocyte count and hematocrit:

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

With expected maturation time as follows:

Days	Hematocrit
1	45%
1.5	35%
2	25%
3	15%

A reticulocyte production index greater than or equal to 3 is considered normal, while an index of less than 2 is below normal.²



If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Brecher G: New methylene blue as a reticulocyte stain. Am J Clin Pathol 19:895, 1949
2. National Committee for Clinical Laboratory Standards. Method for Reticulocyte Counting, Proposed Standard. H16-P, Vol 5, No. 10, 1985
3. Perrotta AL, Finch CA: The polychromatophilic erythrocyte. Am J Clin Pathol 57:471, 1972

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Procedure No. R 4132
Previous Revision: 2003-03
Revised: 2003-09

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