

PRODUCT SPECIFICATION SHEET

RETICULOCYTE DILUTING FLUID (AR038)

INTRODUCTION

Reticulocytes are juvenile red cells that pass into the blood stream from the bone marrow. Reticulocytes stay in circulation for about 24 hours and mature into erythrocytes. Reticulocytes have ribosomal and cytoplasmic remnants which picks up supravital stains when the stain is allowed to penetrate the cells while in the living condition. Following this process of supravital staining, a blood smear is made and the number of reticulocytes are counted against the number of red cells observed in the blood smear.

PRINCIPLE

The number of reticulocytes (abbreviated as retics) in the blood circulation indicates the degree of activity of bone marrow; and when the marrow is very active (e.g. in haemolytic anaemia or acute blood loss) their number increases. This is known as reticulocytosis. In case of aplastic anaemia (poor activity of bone marrow) the reticulocyte count is decreased.

FORMULA

Ingredients	Formula / Litre
New Methylene Blue	1.0 gm
Sodium Citrate	0.4 gm
Salinated Distilled Water	100 ml

REAGENT STORAGE AND STABILITY

- 1. Store the reagents at 2-8 °C. DO NOT FREEZE
- 2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

PRECAUTIONS

- 1. For Invitro Diagnostic use only.
- 2. Observe all standard safety precautions consistent with hazard(s) stated
- 3. Avoid contact with eyes, skin, or mucous membranes. If contact occurs, wash immediately with copious amounts of water. The reagent has corrosive and flammable liquids; keep away from open flame.

SPECIMEN COLLECTION

EDTA-anticoagulated blood is commonly used but other anticoagulants do not interfere. Capillary blood can also be used (heparinized). A blood specimen collected within 2 to 3 hours should be used; older blood specimens show artifacts.

PROCEDURE

- Transfer two drops of the stain and two drops of well-mixed blood specimen into a small test tube (5ml) with the help of two separate Pasteur pipettes. If capillary blood is used (non-anticoagulated), transfer the blood directly into the test tube. The blood will not clot because the stain contains the anticoagulant.
- 2. Mix the NMB stain and the blood specimen; cover the test tube with a cork or aluminium foil to prevent evaporation. Put the timer on or note the time.
- 3. Leave the test tube undisturbed for 15 minutes at room temperature.
- 4. After 15 minutes, mix the contents of the tube and remove one small drop of the mixture to a clean grease-free slide (clean with alcohol-soaked surgical gauze and then polish with dry gauze). Use of a capillary tube or a pair of applicator sticks is recommended for getting a tiny drop of the specimen. A bigger drop will not give a good smear. A Pasteur pipette can be used if the opening is very narrow like the capillary tube.
- 5. Prepare a thin smear of the stained blood specimen with the help of a spreader slide.





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- 6. Air dry the smear.
- 7. Examine the smear first under the low power objective for scanning, and locate a thin portion of the smear where the red cells are evenly distributed and are not touching each other.
- 8. Carefully change to the oil-immersion objective (100x), focus sharply and try to locate an area in which there are approximately 100 to 150 red cells visible in the oil-immersion field.

OBSERVATIONS

Reticulocytes are identified by the fine, deep violet filaments and granules arranged in a network. Red cells stain pale blue. Examine at least 15 fields having 100 to 150 red cells in each field. Simultaneously enumerate the reticulocytes and red cells in each field. Use a hand tally counter, if available. Counting is easier if the size of the microscopic field is reduced. This can be done by placing in the eye-piece a small circular piece of black paper (or index card) in which a hole of 5 mm diameter has been made with a puncher.

NORMAL VALUE

Adults: 0.2 - 2% Infants: 2 - 6%

CALCULATIONS

Calculate the reticulocyte count as follows:

No. of red cells examined

SOURCES OF ERROR

- 1. Staining time should not be less than 10 minutes.
- 2. Mix the blood and the stain gently thoroughly prior to making the smear. This is important. The reticulocytes have a lower specific gravity than mature red cells and therefore settle on top of the red cells in the mixture. Thus an unmixed or poorly mixed blood specimen may not give the true picture.
- 3. Red cells showing highly refractile areas may be confused with reticulocytes. These artifacts in the red cells are probably due to moisture in the air and poor drying of the smear. Use fresh specimen.

USER QUALITY CONTROL

Check signs of deterioration. Check the performance of the reagent weekly using 18-24 hour standard ATCC cultures of known indole-positive and indole -negative microorganisms. The following test strains are recommended:

PACKAGING

Product Name : **Reticulocyte Diluting Fluid** Product Code : **AR038** Available Pack sizes : **25ml**

FURTHER INFORMATION

For further information please contact your local MICROMASTER Representative.





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MICROMASTER LABORATORIES PRIVATE LIMITED

AR038PSS, QAD/FR/024, Rev.00

Unit 38/39, Kalpataru Industrial Estate, Off G.B. Road, Near 'R-Mall', Thane (W) – 400607. M.S. INDIA. Ph: +91-9320126789/9833630009/9819991103 Email: <u>sales@micromasterlab.com</u>

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