

B-158 BRILLIANT CRESYL BLUE STAIN FOR RETICULATED CELLS AND PLATELETS

Fixation: None

Staining:

1. Polish smooth glass slides or cover glasses.
2. Place a drop of **Alcoholic Brilliant Cresyl Blue, 0.3%** (B-158-1), on the glass and allow to dry.
3. On a second, clean slide or cover glass, place a drop of blood 2-3mm. in diameter, and bring this into contact with the dried stain (step. 2).
4. Move the slides or covers (hinge fashion, up or down) until all the stain is dissolved and the blood appears blue-black. Then allow the glasses to come in contact so as to spread the drop.
5. Separate the glasses and allow films to dry.
6. Place 1 ml of **Wright Staining Solution** (B-158-2), on the blood film for 1-3 minutes.
7. Add 2 ml of distilled water or preferable **Phosphate Buffer, pH 6.5** (B-158-3) and let stand for twice the staining time used in step 6. Flood off the stain and wash with distilled water or preferably **Phosphate Buffer, pH 6.5** (B-158-3), until the thin portions of the stained film are pink.
8. Dry by blotting carefully. If poor staining results, try varying the staining time with **Wright's Stain** (B-158-2), either before or after dilution.

Note: Record the number of reticulocytes and/or platelets noted in counting 1000 or more red blood cells. The number of red cells per cubic millimeter should be determined in haemocytometer, and the ratio of the reticulocytes or platelets to red cells computed from the stained preparations.

Stain Results:

Reticulum of immature red cells	Clear-cut blue
Background	Pale-blue or eosin colored
Blood Platelets	Lilac

References:

- Cunningham, R.S., Arch. Int. Med., 26:405-9, 1920.
McClung, C.E., Microscopical Technique, 2nd edition, Paul B. Hoeber, Inc. N.Y., p. 319., 1937.
Clark, G., (ed.), Staining Procedures, 3rd ed., Williams & Wilkins, Baltimore, P. 128, c1973
Wright, J.H., J. Med. Res., 7: 138-144., 1902.
Conn., H.J., Biological Stains, 4th ed. Biotech Publications, Geneva, N.Y., pp. 172-173., 1940.

