INTENDED USE

Gram Stain reagents are intended for use in the delineation of Gram-Positive and Gram-Negative organisms in films and tissue. Gram Stain reagents are for “In Vitro Diagnostic Use”.

Gram stain is used clinically to delineate two distinct groups of microorganisms. Those which retain primary dye (crystal violet) are called Gram positive. Those which lose the primary dye during a decolorization step are called Gram negative. The mechanisms whereby Gram positive organisms retain the primary stain are unknown, although the chemistry and structure of cell walls are most certainly involved.

Numerous modifications of the original Gram method have been described. The Sigma-Aldrich procedure is based on the work of Hucker and Conn utilizing a crystal violet-ammonium oxalate solution that aids in differentiation and is quite stable.

REAGENTS

CRYSTAL VIOLET SOLUTION, Catalog No. HT901-8 fl oz Certified crystal violet, 2.31%, ammonium oxalate, 0.1%, and 20% ethyl alcohol, SD3A.

GRAM’S IODINE SOLUTION, Catalog No. HT902-8 fl oz Iodine, 0.33%, and potassium iodide, 0.66%.

DECOLORIZER SOLUTION, Catalog No. HT903-8 fl oz Required for films only. Isopropyl alcohol, 75%, and acetone, 25%.

SAFRANIN O SOLUTION, Catalog No. HT904-8 fl oz Certified safranin 0.6% in 20% ethyl alcohol, SD3A.

TARTRAZINE SOLUTION, Catalog No. HT3028-250 ml Required for tissue only. Tartrazine, 0.25%, and acetic acid, 0.25%.

STORAGE AND STABILITY:
Store at room temperature (18–26°C). Reagent label bears the expiration dating. Use once and discard.

PREPARATION:
All reagents are ready to 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 and product labeling for any updated risk, hazard or safety information.

PROCEDURE FOR TISSUE:

1. Deparaffinize sections and hydrate to deionized water.
2. Place slides on staining rack and cover sections with Crystal Violet Solution, for 1 minute.
3. Drain off Crystal Violet Solution and rinse thoroughly in deionized water.
4. Mordant in Gram’s Iodine Solution for 5 minutes.
5. Rinse in deionized water and blot sections.
6. Differininate in absolute alcohol or acetone.
7. Rinse in deionized water.
8. Cover slides with Safranin O Solution for 30-60 seconds.
9. Rinse in deionized water and blot sections.
10. Cover sections with Tartrazine Solution for 5-10 seconds.
11. Blot off excess stain.
12. Rinse in 2 changes absolute alcohol.
13. Clear in xylene and mount.

PERFORMANCE CHARACTERISTICS

Gram positive organisms — Purple
Gram negative organisms — Red

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

REFERENCES

1. Gram C; Ueber die isolierte färbung der schizomyzeten in schnitt und trockenpräparaten. Fortschr Med 2:185, 1884
2. Hucker GJ, Conn HJ; Further studies on the methods of Gram staining. NY Agric Exp Stn Tech Bull No. 128, 1927

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