SIGMA-ALDRICH®

GRAM STAIN
(Procedure No. HT90)

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[1] have been described. The Sigma-Aldrich procedure is based on the work of Hucker and Conn[2] 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, 2.3%, 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.

Gram Stain TISSUE-TROL™ control slides are paraffin embedded animal tissue containing gram negative and gram positive bacteria and should be considered potentially infectious.

PROCEDURE

SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. 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.

Films: Any well prepared, heat-fixed film is acceptable. The thickness of the film will influence the time required for decolorization.

Tissue: Any well fixed paraffin embedded tissue cut at 5 microns.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
Positive control slides should be included in each run, Gram Stain TISSUE-TROL™ ,
Catalog No. G3045 or TTR005.
Alcohol, Absolute
Acetone, ACS Grade
Xylene or xylene substitute
Microscope

NOTES:
Care should be taken to avoid over decolorization of the slides with the Decolorizer Solution. Even Gram positive organisms will become colorless and appear red if the Decolorizer solution is left on the slide for excessive periods of time.[4]

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:
PROCEDURE FOR FILMS:
1. Flood film with Crystal Violet Solution for **1 minute**.
2. Rinse thoroughly in deionized water.
3. Flood film with Gram's Iodine Solution for **1 minute**.
4. Rinse thoroughly in deionized water.
5. Flood with Decolorizer Solution for up to **10 seconds** until color stops running.
6. Rinse thoroughly in deionized water.
7. Flood film with Safranin O Solution for **1 minute**.
8. Rinse thoroughly in deionized water.
9. Air dry film and examine microscopically.

PROCEDURE FOR TISSUE:[3]
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. Differentiate 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

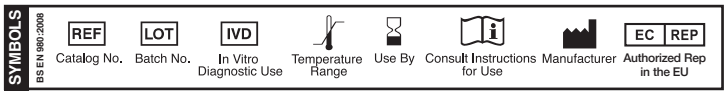
Gram negative organisms — Red

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

REFERENCES

1. Gram C: Ueber die isoliete färbung der schizomyceten 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
3. Histopathological Technic and Practical Histochemistry, 3rd ed., RD Lillie, Editor. McGraw-Hill, New York, 1965, pp 565-567
4. Theory and Practice of Histotechnology, 2nd Edition, Sheehan DC and Hrapchak BB, Battelle Press, Columbus (OH), 1980 p 234

Procedure No. HT90
Previous Revision: 2014-09
Revised: 2016-11



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