
INTENDED USE

Sigma-Aldrich 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 method¹ have been described. The Sigma-Aldrich procedure is based on the work of Hucker and Conn² utilizing a crystal violet-ammonium oxalate solution that aids in differentiation and is quite stable.

REAGENTS

CRYSTAL VIOLET SOLUTION, Catalog No. HT90-1

Certified crystal violet, 2.3%, ammonium oxalate, 0.1%, and 20% ethyl alcohol, SD3A.

GRAM'S IODINE SOLUTION, Catalog No. HT90-2

Iodine, 0.33%, and potassium iodide, 0.66%.

DECOLORIZER SOLUTION, Catalog No. HT90-3

Required for films only.

Isopropyl alcohol, 75%, and acetone, 25%.

SAFRANIN O SOLUTION, Catalog No. HT90-4

Certified safranin 0.6% in 20% ethyl alcohol, SD3A.

TARTRAZINE SOLUTION, Catalog No. HT30-2-8

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 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.

US Risks and Safety Statements

Crystal Violet Solution is COMBUSTIBLE and an IRRITANT. Irritating to respiratory system and skin. Risk of serious damage to eyes. Limited evidence of a carcinogenic effect. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. Avoid releases to the environment. Refer to special instructions/safety data sheets.

Gram's Iodine Solution. Caution: Substance not yet fully tested.

Decolorizer Solution is FLAMMABLE and an IRRITANT. Highly flammable. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Keep container tightly closed and in a well-ventilated place.

Safranin O solution is FLAMMABLE and HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Reagent Alcohol is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

Xylene is FLAMMABLE and HARMFUL. Possible risk of impaired fertility. May cause harm to the unborn child. Harmful by inhalation and in contact with skin. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Acetone is FLAMMABLE and an IRRITANT. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Target organs: Liver and kidneys.

EU Risks and Safety Statements (Caution: Substances not yet fully tested)

Crystal Violet Solution is HARMFUL. Flammable. Limited evidence of a carcinogenic effect. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Keep away from sources of ignition - no smoking. Wear suitable protective clothing and gloves. Avoid releases to the environment. Refer to special instructions/safety data sheets.

Gram's Iodine Solution. Caution: Substance not yet fully tested.

Decolorizer Solution is FLAMMABLE and an IRRITANT. Highly flammable. Irritating to respiratory system and skin. Risk of serious damage to eyes. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Safranin O solution is FLAMMABLE. Keep away from sources of ignition - no smoking.

Reagent Alcohol is HIGHLY FLAMMABLE and an IRRITANT. Highly Flammable. Irritating to eyes, respiratory system and skin. Keep container tightly closed. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Xylene is HARMFUL. Flammable. Harmful by inhalation and in contact with skin. Irritating to skin. Avoid contact with eyes.

Acetone is HIGHLY FLAMMABLE and an IRRITANT. Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness. Keep container in a well-ventilated place. Keep away from sources of ignition - no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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.

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

ALCOHOL, ABSOLUTE

ACETONE, ACS GRADE

XYLENE

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, Catalog No. HT90-1, for **1 minute**.
2. Rinse thoroughly in deionized water.
3. Flood film with Gram's Iodine Solution, Catalog No. HT90-2, for **1 minute**.
4. Rinse thoroughly in deionized water.
5. Flood with Decolorizer Solution, Catalog No. HT90-3, for up to **10 seconds** until color stops running.
6. Rinse thoroughly in deionized water.
7. Flood film with Safranin O Solution, Catalog No. HT90-4, 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, Catalog No. HT90-1, for **1 minute**.
3. Drain off Crystal Violet Solution and rinse thoroughly in deionized water.
4. Mordant in Gram's Iodine Solution, Catalog No. HT90-2, 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, Catalog No. HT90-4, for **30-60 seconds**.
9. Rinse in deionized water and blot sections.
10. Cover sections with Tartrazine Solution, Catalog No. HT30-2 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 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

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