

65092-93**Microscopy****Harleco® Gram Stain Set**

Complete bacterial staining kit containing crystal violet, iodine, decolorizer and safranin stain



In Vitro Diagnostic Medical Device

Intended Use

Harleco® Gram stain set is used to qualitatively assess the differential staining of bacteria from cultures and human specimens by the modified Gram stain method.

Principle

Differential staining of bacterial cells in tissue by the Gram method was first mentioned in a treatise by Cad Freidlander,³ in 1883. In 1884, Christian Gram⁴ published a detailed account of his staining procedure. Three of the various modifications were found to give superior results: Hucker,^{1,2} Burke,⁶ and Kopeloff-Beerman.⁷

Our method uses the Gram technique except that the ethanol decolorizer solution has been replaced by an isopropanol/acetone decolorizer solution. The function of the isopropanol in the acetone is to decrease the rate of decolorization.² Both Gram-positive and Gram-negative cells take up the primary crystal violet stain. The addition of iodine causes the formation of an iodine-crystal violet complex within the cell wall.

Upon the application of decolorizer, lipid is extracted from the cell walls of Gram-negative bacteria, and the cell wall porosity is increased.^{8,9} The iodine-crystal violet complex then diffuses from the cell.

Simultaneously, Gram-positive bacterial cells are dehydrated, cell wall pore size is decreased, and the iodine-crystal violet complex is trapped within the cell.^{8,9}

The increased porosity of the Gram-negative cells allows the safranin counterstain to enter the bacteria.

Sample material

Immediately upon obtaining specimen, prepare slide as indicated. Once prepared, the specimen may be stored indefinitely at room temperature in appropriate containers e.g., slide boxes.

Cultured Specimens**Liquid Media:**

1. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
2. Dip loop into suspension and spread on clean glass slide.
3. Air dry smear and heat fix by flaming with a Bunsen burner; allow slide to cool before staining.

Solid Media:

1. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
2. Place loop of ultrapure water on a clean glass slide.
3. Sterilize wire loop by flaming with a Bunsen burner; allow to cool.
4. Touch needle to colony, suspend in water droplet, and spread evenly on slide.
5. Air dry smear and heat fix by flaming with a Bunsen burner; allow slide to cool before staining.

Human Specimens:

1. Complete explanation of collection of specimens from patients can be found in the Manual of Clinical Microbiology.¹⁰
2. Obtain specimen with sterile loop, swab or pipette.
3. Smear specimen on a clean glass slide, air dry, and heat fix; allow slide to cool before staining.

Reagents

Cat. No. 65092A
Harleco® Crystal Violet Solution Gram 500 mL
Crystal violet, 100% P.D.C.* (3.5 g/L)
SDA-3A Alcohol,
95% Ethyl, 5% Methyl Alcohol (200 mL/L)
Preservative

WARNING! CAUSES EYE IRRITATION. Avoid contact with eyes. Wash thoroughly after handling. **COMBUSTIBLE.** Keep away from heat and open flame.

FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

Cat. No. 65092B
Harleco® Safranin Stain 500 mL
Safranin O, 100% P.D.C. (6.04 g/L)
SDA-3A Alcohol
95% Ethyl, 5% Methyl Alcohol (200 mL/L)

WARNING! COMBUSTIBLE. Keep away from heat and open flame.

Cat. No. 65092D
Harleco® Gram Iodine Solution 500 mL
PVP Iodine (130 g/L)
Potassium Iodide (19 g/L)

WARNING! CAUSES EYE IRRITATION. Avoid contact with eyes. Wash thoroughly after handling.

FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

Cat. No. 65092E
Harleco® Decolorizer 500 mL
Acetone (200 mL/L)
Isopropyl Alcohol 99% (800 mL/L)

DANGER! EXTREMELY FLAMMABLE.
CAUSES EYE IRRITATION: Keep away from heat, sparks, and open flame. Keep container closed. Use with adequate ventilation. Avoid contact with eyes. Wash thoroughly after handling.

FIRST AID: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

*Pure Dye Content

Also required:

Cat. No. 64969
Harleco® Krystalon™ Mounting Medium

Cat. No. 104699
Immersion oil for microscopy

Sample preparation

The sampling must be performed by qualified personnel. All samples must be clearly labeled. Suitable instruments must be used for collecting and preparing samples. Follow the manufacturer's instructions for application/use.

Reagent preparation

All solutions provided in the kit are ready-to-use.

Procedure

Materials Provided

65092A Crystal Violet Solution Gram
65092B Safranin Stain
65092D Gram Iodine Solution
65092E Decolorizer

Materials Required But Not Provided

Wire loop or inoculating needle
Bunsen burner
Glass slides
Cover slips
Timer accurate to 15 seconds per minute
Absorbent paper
Immersion oil
Mounting media
Microscope, with oil immersion lens

Procedure Outline

1. Flood prepared slide with Crystal Violet Solution Gram. Allow to stand one minute.
2. Rinse gently with distilled water.
3. Flood slide with Gram Iodine Solution: allow to stand one minute.
4. Rinse gently with distilled water.
5. Decolorize specimen by rinsing with Decolorizer for 10 seconds. Decolorization is complete when solution runs colorless from slide.
6. Wash gently, but thoroughly, with distilled water.

7. Flood slide with Safranin Stain; allow to stand 1 minute.
8. Rinse gently with distilled water.
9. Blot with absorbent paper.
10. Examine prepared specimen under oil immersion lens.

Stability of Prepared Slide

Once the Gram stain has been performed, the resulting slide is stable indefinitely, particularly if covered with a glass coverslip and mounting medium.

Quality Control

To check the stability of the reagents and stains, perform Gram stains on an 18 to 24 hr. old culture of *S. aureus* and *E. coli*. *S. aureus* yields Gram-positive, purple cocci, and *E. coli* yields Gram-negative, pink to red rods. If these results are not obtained the following checks should be made:

1. Stain a prepared slide of 18 to 24 hr. old cultures of *S. aureus* with Crystal Violet Solution Gram. Rinse gently with cold water. (Gram-positive, purple cocci should be observed; if not, discard stain.)
2. Stain a prepared slide of 18 to 24 hr. old cultures of *E. coli* with Safranin Stain. Rinse gently with cold water. (Gram-negative, pink to red rods should be observed; if not, discard stain.)
3. Stain a prepared slide of 18 to 24 hr. old cultures of *S. aureus* with Crystal Violet Solution Gram. Rinse gently with cold water. Flood slide with Gram Iodine Solution and allow to stand for 1 minute. Decolorize by rinsing with Decolorizer for 10 seconds or until solution runs colorless. At this point, the specimen should be purple; if not, the Gram Iodine Solution is not forming an iodine-crystal violet complex and should be discarded.

Result

Gram-positive --- purple

Gram-negative --- pink to red

Technical notes

Heat fixing: In order to prevent cell wall eruption, care must be taken not to heat too long or vigorously. If the temperature of the slide becomes hot enough to burn human skin, the cellular structure may become damaged.

Decolorization: Gram-positive bacteria can be decolorized if Decolorizing Solution is applied much in excess of 10 seconds. Close adherence to the recommended procedure is necessary.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel. Valid nomenclature must be used.

Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application in order to avoid an incorrect result.

Storage

The Gram Stain Set reagents should be stored in their original containers at room temperature (15-25 °C). Storage must not exceed expiration date on box label. Precautions should be taken to minimize exposure to air and heat because the Gram Iodine Solution is relatively unstable and may show a decrease in iodine concentration over time. It is therefore important that controls be included in the daily work regime to ensure that the iodine solution is providing the proper mordant activity.

Indications of Deterioration

Some precipitation may occur in the Crystal Violet Solution Gram. If it appears to affect staining results, warm in a 37 °C incubator, then shake until precipitate is dissolved. Refer to Quality Control section for additional information.

Shelf-life

The Gram Stain Set can be used until the stated expiration date on the packaging. After first opening of the bottle, the contents can be used up to the stated expiry date when stored at 15-25 °C.

Additional instructions

For professional use only.

The application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standards must be used.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Auxiliary reagents

Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	50 mL, 500 mL
Cat. No. 104699	Immersion oil for microscopy	100 mL, 500 mL

Hazard classification

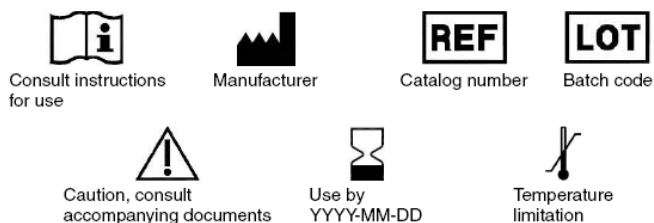
Cat. No. 65092 Please observe the hazard classification printed on the label and the information given in the safety data sheet. The safety data sheet is available on the website and on request.

Literature

1. Hucker, G.J. J. Bacteriol. 6:395; 1921
2. Hucker, G.J. and Conn, N.J., NY Agr. Exp. Sta. Tech. Bull. 128:1, 1927
3. Friedlander, C. Fortschr. Med. 1:715; 1883
4. Gram, C. Fortschr. Med. 2:185; 1884
5. Hucker, G.J. Abstr. Bacteriol. 8:2; 1922
6. Burke, V.J. Bacteriol. 7:159; 1922
7. Kopeloff, N., and Beerman, P.J. Infect. Diseases. 31:480; 1922
8. Peiczar, M.J. Jr, and Reid, M.D. Microbiology, 2nd ed. New York; McGraw-Hill; 50-52; 1965
9. Bartholomew, J.W. and Mittwer, T.J. Bacteriol. 18:1; 1952
10. Manual of Clinical Microbiology of the American Society for Microbiology, 30-45; 1970

Replacement items for this set:

65092A-95	Crystal Violet Solution Gram	4x500 mL
65092B-95	Safranin Stain	1x500 mL
65092D-95	Gram Iodine Solution	1x500 mL
65092E-95	Decolorizer	1x500 mL
65092A-85	Crystal Violet Solution Gram	4 L
65092B-85	Safranin Stain	4 L
65092D-85	Gram Iodine Solution	4 L
65092E-85	Decolorizer	4 L



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