remel

GRAM STAIN KIT, ENHANCED

INTENDED USE
Remel Gram Stain Kit, Enhanced is recommended for use in qualitative procedures to differentiate gram-negative from gram-positive organisms.

SUMMARY AND EXPLANATION
The Gram stain method was developed in 1884 by the Danish bacteriologist, Christian Gram, to differentiate bacterial cells from infected tissue.[1-3] Later it was discovered that the bacterial cell wall composition was the key to the Gram stain reaction, in that it separates bacteria into two groups based on cell color. Over the years there have been many modifications of the original technique; however, the Gram stain is still the most common and cost-effective staining technique used in identifying bacteria.

PRINCIPLE
Gram staining is based on the ability of the bacterial cell wall to retain crystal violet dye during decolorizing treatment with a decolorizing agent. Gram Crystal Violet, Enhanced is the primary stain taken up by all bacteria due to its ability to rapidly permeate the cell wall and stain the protoplast purple. Gram Iodine, Enhanced is added as a fixative to form a complex with the primary stain so the dye cannot be easily removed. Gram Decolorizer, Enhanced, a mixture of ethanol and acetone, is added in the third step. The cell walls of gram-positive organisms have a thick peptidoglycan layer and large amounts of teichoic acids. As a result, they are unaffected by alcohol decolorization, retain the crystal violet-iodine complexes, and appear purple in color.[4] The cell walls of gram-negative organisms have an outer membrane which is damaged by the alcohol decolorizer, allowing the crystal violet-iodine complex to leak out and be replaced by the counterstain. Gram Iodine, Enhanced is a modification of the original iodine formulation; sodium bicarbonate has been added and the iodine concentration increased.[5,6] Gram Enhancer is used to reduce the red color of background material and improve the differentiation of gram-negative bacteria from the background. Gram Safranin, Enhanced is the counterstain which stains gram-negative cells red. Depending on the source material, the background stain will appear from rust or light violet to blue-green.

REAGENTS (CLASSICAL FORMULA)*

GRAM CRYSTAL VIOLET, ENHANCED

Ammonium Oxalate (CAS 6009-70-7)	9.4 g
Crystal Violet (CAS 548-62-9)	4.0 g
Ethyl Alcohol 95% (CAS 64-17-5)	80.0 ml
Demineralized Water (CAS 7732-18-5)	920.0 ml

GRAM IODINE, ENHANCED

Potassium Iodide (CAS 7681-11-0)	8.0 g
Iodine (CAS 7553-56-2)	4.0 g
Sodium Bicarbonate (CAS 144-55-8)	0.5 g
Demineralized Water (CAS 7732-18-5)	1000.0 ml

GRAM DECOLORIZER, ENHANCED

Ethyl Alcohol (CAS 64-17-5)	600.0 ml
Acetone (CAS 67-64-1)	400.0 ml

GRAM ENHANCER

Fast Green (CAS 2353-45-9)	0.3 g
Tartrazine (CAS 1934-21-0)	0.01 g
Formaldehyde (CAS 50-00-0)	20.0 ml
Demineralized Water (CAS 7732-18-5)	1000.0 ml

GRAM SAFRANIN, ENHANCED

Safranin (CAS 477-73-6)	2.5 g
Sodium Acetate (CAS 127-09-3)	0.1 g
Ethyl Alcohol 95% (CAS 67-17-5)	25.0 ml
Demineralized Water (CAS 7732-18-5)	975.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
Gram Stain Kit, Enhanced is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

GRAM CRYSTAL VIOLET, ENHANCED
CAUTION! COMBUSTIBLE, keep away from heat and flame. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Wash thoroughly after handling. Call a physician if swallowed.

GRAM IODINE, ENHANCED
Warning! Causes irritation and possible burns by all routes of exposure. May cause allergic skin reaction. May be harmful if swallowed or absorbed through the skin. This substance has caused adverse reproductive and fetal effects in animals.

GRAM DECOLORIZER, ENHANCED
CAUTION! Flammable, keep away from heat, sparks, and flame. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Wash thoroughly after handling. Call a physician if swallowed.

GRAM ENHANCER
DANGER! May be harmful or fatal if swallowed. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Wash thoroughly after handling. Call a physician if swallowed.

GRAM SAFRANIN, ENHANCED
CAUTION! COMBUSTIBLE, keep away from heat and flame. May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Wash thoroughly after handling. Call a physician if swallowed.

STORAGE
This product is ready for use and no further preparation is necessary. Store the product in its original container at 20-25°C until used.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE AND TRANSPORT
Specimens should be collected and handled following recommended guidelines.[4,7]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Glass slides, (7) Bunsen burner or slide warmer, (8) Microscope, immersion oil, (9) Methanol, (10) Staining rack, (11) Demineralized water.

PROCEDURE
Note: Immediately before use, add the (supplied) Gram Iodine, Enhanced concentrate to the bottles of supplied diluent.

1. Make a thin smear of the material for study. Allow the slide to air-dry completely or use a slide warmer. Fix the slide by passing 3 or 4 times through the flame of a Bunsen burner or flood the smear with methanol and allow it to evaporate.
2. Place the slide on a staining rack and overlay with Gram Crystal Violet, Enhanced for 5-10 seconds.
3. Wash thoroughly with demineralized water and overlay with Gram Iodine, Enhanced for 10 seconds.
4. Flood with Gram Decolorizer, Enhanced until the solvent flows colorless from the slide (10-30 seconds).
5. Flood with Gram Enhancer for 3 seconds.
6. Rinse with demineralized water and overlay with Gram Safranin, Enhanced for 15 seconds.
7. Rinse with demineralized water and allow the slide to air dry.

INTERPRETATION OF THE TEST

Gram-positive organisms stain purple.
Gram-negative organisms stain red.

QUALITY CONTROL

All lot numbers of Gram Stains Enhanced have been tested and found to yield acceptable stain results as listed in the **Interpretation** section. Positive and negative control slides should be tested prior to use of new lot numbers of stains and decolorizing reagent and at least weekly thereafter. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. Test positive and negative controls with each new lot number of stains and weekly thereafter to verify reagent efficacy and ensure the decolorization procedure is performed correctly.[4]
2. To obtain the most reliable results, remove clinical specimen isolates from an 18-24 hour culture growing on nonselective medium.[4]
3. Do not overheat smears when fixing slides. Excessive heating may cause atypical staining.[4,8]
4. Gram-positive organisms contained in a specimen may appear gram-negative if the patient is on antimicrobial therapy.[4]
5. Precipitated crystal violet can appear as coccoid shapes or fungal elements, as well as other artifacts or background material.[4]

BIBLIOGRAPHY

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3. Bartholomew J.W. and T. Mittwer. 1951. Stain Technol. 26:231-240.
4. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed., American Society for Microbiology, Washington, D.C.
5. Biswas, B.B., P.S. Basu, and M.K. Pal. 1970. Int. Rev. Cytol. 29:1-27.
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7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2007. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, St. Louis, MO.
8. Mangels, J.I., M.E. Cox, and L.H. Lindberg. 1984. Diagn. Microbiol. Infect. Dis. 2:129-137.

PACKAGING

GRAM CRYSTAL VIOLET, ENHANCED:
REF R40225, 250 ml/Bottle.....5/Pk
REF R40226, Gallon.....Each

GRAM IODINE, ENHANCED:
REF R40234, 250 ml/Bottle.....5/Pk
REF R40235, Gallon.....Each

GRAM DECOLORIZER, ENHANCED:
REF R40228, 250 ml/Bottle.....5/Pk
REF R40229, Gallon.....Each

GRAM ENHANCER:
REF R40231, 250 ml/Bottle.....5/Pk
REF R40232, Gallon.....Each

GRAM SAFRANIN, ENHANCED:
REF R40237, 250 ml/Bottle.....5/Pk
REF R40238, Gallon.....Each

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

CAS (Chemical Abstracts Service Registry No.)

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