



## GRAM STAIN KIT & REAGENTS

GRAM STAIN KIT: VGK-200(4 oz.) VGK-400(8 oz.)

SAFRANIN-O:	GRAM'S DECOLORIZER:	GRAM'S IODINE:	CRYSTAL VIOLET:
VGC-008 (8 oz.)	VGD-008 (8 oz.)	VGI-008 (8 oz.)	VGS-008 (8 oz.)
VGC-016 (16 oz.)	VGD-016 (16 oz.)	VGI-016 (16 oz.)	VGS-016 (16 oz.)
VGC-128 (128 oz.)	VGD-128 (128 oz.)	VGI-128 (128 oz.)	VGS-128 (128 oz.)
		VGI-640 (640 oz.)	VGS-640 (640 oz.)

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**SUMMARY AND PRINCIPLES:** In 1883, Karl Friedlander investigated differential staining of bacterial cells in tissue. The following year, Christian Gram published a detailed account of Friedlander's staining procedure. Various modifications of the original Gram's procedure have been proposed over the years. Volu-Sol's Gram Stain is based on the popular Thomas Hucker modification.

Gram staining is a method of differentiating bacterial species into two large groups, gram-positive and gram-negative. The differences in these staining reactions is attributed to the distinctive chemistry and physical structure of the cell walls of these organisms. The differential stain is often described as either related to the higher lipid content of gram-negative bacterial cell walls, or is a magnesium ribonucleate-protein complex reaction. Crystal violet is taken up equally well by both gram-positive and gram-negative bacterial walls. A crystal violet-iodine complex forms in the cell wall of each bacteria after the addition of iodine. After the application of decolorizer lipids are extracted from the cell walls of gram-negative bacteria. This extraction causes an increase in cell wall permeability and results in the loss of the dye complex. Decolorizer dehydrates gram-positive bacteria, decreasing the cell wall permeability, which increases the retention of the crystal violet-iodine complex. The gram-negative bacteria stains pink-red from the safranin-o counterstain.

**SPECIMEN PREPARATION: 1) BACTERIAL GROWTH ON SOLID CULTURE MEDIA:** use a sterile loop or needle to transfer a single colony or mixed growth sample to a small droplet of water on a clean microscope slide. Spread the mixture as required for reading. Allow to dry. **2) BACTERIAL GROWTH IN LIQUID MEDIA:** Use a sterile loop to transfer a portion of the medium to a clean microscope slide. Spread the mixture as required for reading and allow to air dry. **3) BACTERIA IN CLINICAL SPECIMENS:** Depending on specimen type, use either a loop, swab, or pipet to spread the specimen as required for reading, on a clean microscope slide. Allow to air dry.

**\*NOTE:** Proper heat fixing is necessary to prevent eruption of cell walls. Excessive heat fixing may alter proper staining reactions. Certain antibacterial drugs can render gram-positive bacteria gram-negative.\*

**PROCEDURE:** Heat fix the slide by passing through a burner flame. Allow to cool. **\*NOTE:** gently rinse the slide with tap or deionized water after each reagent application. And use caution so that slides are not over decolorized, causing gram-positive bacteria to appear gram-negative.\* Cover slide with Volu-Sol's Crystal Violet Reagent for 1 minute. Cover slide with Volu-Sol's Gram's Iodine Reagent for 1 minute. Rinse slide with Volu-Sol's Gram's Decolorizer Reagent until the decolorizer runs off colorlessly. Cover slide with Volu-Sol's Safranin-O Counterstain for 1 minute. Allow slide to drain and air dry, or gently dry with bibulous paper or paper towel. Examine slide under oil immersion lens.

**EXPECTED RESULTS:** Gram-positive cells appear dark violet in color. Gram-negative cells appear pink-red in color. **\*NOTE:** Gram staining variability is common in older specimens and cultures; so it is considered ideal to use cultures after 18-24 hours incubation, and specimens as fresh as possible. It is advisable to check gram stains with specimens of known gram staining reaction, at least on a daily basis (commonly used control specimens are known cultures of Streptococcus or Staphylococcus, and E-coli).\*

**STORAGE AND EXPIRATION:** Store reagents at room temperature (70-77.9 °F/ 20-25.5 °C). Maximum intended shelf life is printed on the label.

**WARNING:** Danger! For in vitro diagnostic use only! Consult a physician if ingested.

### REFERENCES:

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