

**INTENDED USE**

For use in the Gram's Staining method for the initial differentiation of Gram Positive and Gram Negative bacteria.

**SUMMARY AND EXPLANATION**

The Gram stain was originally devised by Christian Gram in 1884. The standard Gram's staining method can be used to differentiate intact, morphologically similar bacteria into two groups. This is based on cell wall colour after employing the staining method. In addition, cell form, size and structural details are evident. This preliminary information can provide initial clues to the type of organism(s) present.

**PRINCIPLE**

A Crystal Violet-Iodine complex forms in the protoplast of all organisms stained using the above procedure. After decolorizing, those organisms that are able to retain this dye complex are classified as Gram positive. Those organisms that are decolorized and take up the counterstain are classified as Gram negative.

Upon disruption or removal of the cell wall, the protoplast of Gram positive as well as Gram negative cells can be decolorized, and hence the Gram negative attribute lost. Therefore, the mechanism of the Gram stain appears to be related to the presence of an intact cell wall able to act as a barrier to decolorization of the primary stain. Generally, the cell wall is non-selectively permeable. It is theorized that during the Gram stain procedure, the cell wall of Gram positive cells is dehydrated by the alcohol in the decolorizer and loses permeability, hence it retains the primary stain. In the case of the cell wall of the Gram negative cells, due to a higher lipid content, the cell wall becomes more permeable when treated with alcohol, hence the primary stain is lost, allowing for the later counterstain to be taken.

**REAGENTS**
**Ready to use stains.**

PL7000	Crystal Violet	500 ml
PL7001	Crystal Violet	1 litre
PL7002	Crystal Violet	2 litre
PL7000/25	Crystal Violet	250 ml
PL7003	Gram's Iodine	500 ml
PL7004	Gram's Iodine	1 litre
PL7005	Gram's Iodine	2 litre
PL7003/25	Gram's Iodine	250 ml
PL7006	Gram's Differentiator	500 ml
PL7007	Gram's Differentiator	1 litre
PL7008	Gram's Differentiator	2 litre
PL7006/25	Gram's Differentiator	250 ml
PL7009	Neutral Red	500 ml
PL7010	Neutral Red	1 litre
PL7011	Neutral Red	2 litre
PL7009/25	Neutral Red	250 ml
PL7012	Safranin	500 ml
PL7013	Safranin	1 litre
PL7014	Safranin	2 litre
PL7012/25	Safranin	250 ml
PL7015	Dilute Carbol Fuchsin	500 ml

PL7016	Dilute Carbol Fuchsin	1 litre
PL7017	Dilute Carbol Fuchsin	2 litre
PL7015/25	Dilute Carbol Fuchsin	250 ml
PL7052	Lugol's Iodine	500 ml
PL7053	Lugol's Iodine	1 litre
PL7053-2	Lugol's Iodine	2 litre
PL7101	Basic Fuchsin / Neutral Red	500 ml
PL7102	Basic Fuchsin / Neutral Red	1 litre
PL7103	Basic Fuchsin / Neutral Red	2 litre
PL7073	CV - Ammonium Oxalate	500 ml
PL7074	CV - Ammonium Oxalate	1 litre
PL7075	CV - Ammonium Oxalate	2 litre
PL7110	Sandifords Stain	500 ml
PL7111	Sandifords Stain	1 litre
PL7112	Sandifords Stain	2 litre
PL7113	Methyl Violet	500 ml
PL7114	Methyl Violet	1 litre
PL7115	Methyl Violet	2 litre
PL7116	Safranin / Neutral Red	500 ml
PL7117	Safranin / Neutral Red	1 litre
PL7118	Safranin / Neutral Red	2 litre

**Concentrated Stains. Dilute to 1 litre with distilled water before use.**

PL8000	Crystal Violet	100 ml
PL8001	Gram's Iodine	100 ml
PL8002	Neutral Red	100 ml
PL8003	Safranin	100 ml
PL8004	Dilute Carbol Fuchsin	100 ml
PL8010	Lugol's Iodine	100 ml
PL8011	Methyl Violet	100 ml

**Concentrated Stains. Dilute to 4 litres with distilled water before use.**

PL8000-4.0	Crystal Violet	400 ml
PL8001-4.0	Gram's Iodine	400 ml
PL8002-4.0	Neutral Red	400 ml
PL8003-4.0	Safranin	400 ml
PL8004-4.0	Dilute Carbol Fuchsin	400 ml
PL8010-4.0	Lugol's Iodine	400 ml
PL8011-4.0	Methyl Violet	400 ml

**Concentrated Stains. Dilute to 5 litres with distilled water before use.**

PL8000-5.0	Crystal Violet	500 ml
PL8001-5.0	Gram's Iodine	500 ml
PL8002-5.0	Neutral Red	500 ml
PL8003-5.0	Safranin	500 ml
PL8004-5.0	Dilute Carbol Fuchsin	500 ml
PL8010-5.0	Lugol's Iodine	500 ml
PL8011-5.0	Methyl Violet	500 ml

**Staining Kits (Ready to use)**

PL8055/25	Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Safranin 250 ml.
PL8056/25	Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Neutral Red 250 ml.
PL8057/25	Gram Staining Kit - Crystal Violet 250 ml, Gram's Iodine 250 ml, Gram's Differentiator 250 ml, Dilute Carbol Fuchsin 250 ml.

**SAFETY PRECAUTIONS**

1. Gram stains from Pro-Lab Diagnostics are offered as an in vitro material and are in no way intended for a curative or prophylactic purpose.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard.
3. The device poses no environmental hazard in excess of those posed by the clinical specimens used with the device. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present. Environmental impact exists and is adequately addressed through proper disposal.

**STABILITY AND STORAGE**

Room Temperature. Away from sources of ignition. Away from direct sunlight. Stored under these conditions, reagents may be used up to the date of expiry on the label.

**SPECIMEN COLLECTION AND PREPARATION OF CULTURES.**

Refer to a standard microbiology text.

**MATERIALS REQUIRED BUT NOT PROVIDED.**

Clean glass slides, sterile loop, flame / hot air, staining rack, tap water, immersion oil, microscope, blotting paper or equivalent substitute.

**PROCEDURE.**

1. Prepare a thin, uniform smear of specimen and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Crystal Violet or Methyl Violet, stand for 1 minute. Rinse with water.
4. Flood the slide with Gram's or Lugol's Iodine, stand for 1 minute. Rinse with water.
5. Gently decolorize with Differentiator for approx. 10 seconds. Rinse with water.
6. Flood the slide with counterstain, stand for 30 – 60 seconds.
7. Rinse well with water, gently blot dry.
8. View using oil immersion microscopy.

**QUALITY CONTROL**

The age of the cultures and the pH of the medium in which the bacteria are grown can markedly affect their reaction to the Gram stain. Use fresh cultures up to 24 hours old.



Recommended QC cultures;

*Escherichia coli* NCTC 10418 (Pink to Red Gram Negative Bacilli)

Oxford *Staphylococcus aureus* NCTC 6571 (Blue to Purple Gram Positive Cocci)

Haemolytic *Streptococcus* Group A NCTC 8198 (Blue to Purple Gram Positive Cocci)

#### INTERPRETATION OF RESULTS

Gram Positive organisms – Blue to Purple.

Gram Negative organisms – Pink to Red.








#### LIMITATIONS

1. False Gram Negative and Gram Positive staining results can be seen due to cellular debris being stained by the technique. e.g. – The nuclei and protoplasm of white blood cells and epithelial cells are stained with counterstain. Solid particulate matter may also be stained by the Crystal Violet.
2. The Gram stain provides preliminary identification information only and is not a substitute for specimen culture.

#### REFERENCES

1. Manual of Clinical Microbiology. Lennette.
2. The Practice of Medical Microbiology. 12th Edition. V2. R. Cruickshank, J. P. Duguid, B. P. Marmion, R.H.A. Swain.

Revision: 2003 12

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= in vitro diagnostic medical device.
	= Temperature limitation
	= Consult instructions for use.