

## INTENDED USE

For use in the Gram staining method for the rapid differentiation of Gram-positive and Gram-negative bacteria in prepared slides from clinical specimens.

## SUMMARY AND EXPLANATION

The Gram stain was originally devised by Hans Christian Gram in 1884. The standard Gram staining method can be used to differentiate intact, morphologically similar bacteria into two groups: Gram positive and Gram negative.

## PRINCIPLE OF THE TEST

Gram positive bacteria have a thicker layer of peptidoglycan in their cell wall which retains the primary stain, appearing purple when finished. Gram negative bacteria allow the primary stain to be flushed away due to a thinner peptidoglycan layer, allowing the final stain to counterstain the bacteria and giving a pink-red appearance when viewed under the microscope.

## MATERIALS PROVIDED

### Ready to use stains and differentiators:

-	PL.7000/100	Crystal Violet	100 ml
-	PL.7000/25	Crystal Violet	250 ml
-	PL.7000	Crystal Violet	500 ml
-	PL.7001	Crystal Violet	1000 ml
-	PL.7002	Crystal Violet	2000 ml
-	PL.7003/100	Grams Iodine	100 ml
-	PL.7003/25	Grams Iodine	250 ml
-	PL.7003	Grams Iodine	500 ml
-	PL.7004	Grams Iodine	1000 ml
-	PL.7005	Grams Iodine	2000 ml
-	PL.7006/100	Grams Differentiator	100 ml
-	PL.7006/25	Grams Differentiator	250 ml
-	PL.7006	Grams Differentiator	500 ml
-	PL.7007	Grams Differentiator	1000 ml
-	PL.7008	Grams Differentiator	2000 ml
-	PL.7009/100	Neutral Red	100 ml
-	PL.7009/25	Neutral Red	250 ml
-	PL.7009	Neutral Red	500 ml
-	PL.7010	Neutral Red	1000 ml
-	PL.7011	Neutral Red	2000 ml
-	PL.7012/100	Safranin	100 ml
-	PL.7012/25	Safranin	250 ml
-	PL.7012	Safranin	500 ml
-	PL.7013	Safranin	1000 ml
-	PL.7014	Safranin	2000 ml
-	PL.7015/100	Dilute Carbol Fuchsin	100 ml
-	PL.7015/25	Dilute Carbol Fuchsin	250 ml
-	PL.7015	Dilute Carbol Fuchsin	500 ml
-	PL.7016	Dilute Carbol Fuchsin	1000 ml
-	PL.7017	Dilute Carbol Fuchsin	2000 ml
-	PL.7052	Lugols Iodine	500 ml
-	PL.7053	Lugols Iodine	1000 ml
-	PL.7053-2	Lugols Iodine	2000 ml
-	PL.7056	Iodine Acetone	500 ml
-	PL.7057	Iodine Acetone	1000 ml
-	PL.7058	Iodine Acetone	2000 ml
-	PL.7073	Crystal Violet - Ammonium Oxalate	500ml
-	PL.7074	Crystal Violet - Ammonium Oxalate	1000ml

-	PL.7075	Crystal Violet - Ammonium Oxalate	2000ml
-	PL.7100/CV	Crystal Violet 0.15%	500ml
-	PL.7101/CV	Crystal Violet 0.15%	1000ml
-	PL.7102/CV	Crystal Violet 0.15%	2000ml
-	PL.7101	Basic Fuchsin / Neutral Red	500ml
-	PL.7102	Basic Fuchsin / Neutral Red	1000ml
-	PL.7103	Basic Fuchsin / Neutral Red	2000ml
-	PL.7106	Grams Differentiator (Acetone/Propan-2-ol)	500ml
-	PL.7107	Grams Differentiator (Acetone/Propan-2-ol)	1000ml
-	PL.7108	Grams Differentiator (Acetone/Propan-2-ol)	2000ml
-	PL.7110	Sandifords Stain	500ml
-	PL.7111	Sandifords Stain	1000ml
-	PL.7112	Sandifords Stain	2000ml
-	PL.7113	Methyl Violet	500ml
-	PL.7114	Methyl Violet	1000ml
-	PL.7115	Methyl Violet	2000ml
-	PL.7116	Safranin / Neutral Red	500ml
-	PL.7117	Safranin / Neutral Red	1000ml
-	PL.7118	Safranin / Neutral Red	2000ml
-	PL.7206/25	Grams Differentiator (Acetone)	250ml
-	PL.7206	Grams Differentiator (Acetone)	500ml
-	PL.7207	Grams Differentiator (Acetone)	1000ml
-	PL.7208	Grams Differentiator (Acetone)	2000ml
-	PL.7306/25	Grams Differentiator (IMS)	250ml
-	PL.7306	Grams Differentiator (IMS)	500ml
-	PL.7307	Grams Differentiator (IMS)	1000ml
-	PL.7308	Grams Differentiator (IMS)	2000ml
-	PL.7406	Grams Differentiator (IMS/Acetone)	500ml
-	PL.7407	Grams Differentiator (IMS/Acetone)	1000ml
-	PL.7408	Grams Differentiator (IMS/Acetone)	2000ml

### Per 100ml solution:

- Ready to use Crystal Violet contains 0.5g of Crystal Violet powder.
- Ready to use Grams Iodine contains 0.38g of Iodine.
- Grams Differentiator contains 50ml of IMS and 50ml of Acetone.
- Ready to use Neutral Red contains 0.4g of Neutral Red powder.
- Ready to use Safranin contains 0.4g of Safranin O.
- Ready to use Dilute Carbol Fuchsin contains 0.15g of Basic Fuchsin powder.
- Ready to use Lugols Iodine contains 0.75g of Iodine.
- Iodine Acetone contains 0.35g of Iodine.
- Crystal Violet Ammonium Oxalate contains 1g Crystal Violet and 0.4g Ammonium Oxalate.
- Crystal Violet 0.15% contains 0.15g of Crystal Violet powder.
- Basic Fuchsin/Neutral Red contains 0.1g Neutral Red and 0.0025g Basic Fuchsin powder.
- Grams Differentiator (Acetone/Propan-2-ol) contains 66.8ml P-2-ol and 33.2ml Acetone.
- Sandifords Stain contains 0.05g of Malachite Green powder and 0.15g of Pyronin Y.
- Ready to use Methyl Violet contains 0.5g of Crystal Violet powder.
- Safranin/Neutral Red contains 0.3g of Safranin O and 0.3g of Neutral Red powder.
- Grams Differentiator (Acetone) contains 98ml of Acetone.
- Grams Differentiator (IMS/Acetone) contains 97ml of IMS and 3ml of Acetone.

### Staining Kits (Ready to use):

- PL.8055/25 Gram Staining Kit (Safranin)  
1 x PL.7000/25, 1 x PL.7003/25, 1 x PL.7006/25, 1 x PL.7012/25

- PL.8056/25 Gram Staining Kit (Neutral Red)  
1 x PL.7000/25, 1 x PL.7003/25, 1 x PL.7006/25, 1 x PL.7009/25
- PL.8057/25 Gram Staining Kit (Dilute Carbol Fuchsin)  
1 x PL.7000/25, 1 x PL.7003/25, 1 x PL.7006/25, 1 x PL.7015/25

### Concentrated Stains (dilute 1 in 10 with deionised or reverse osmosed water before use):

-	PL.8000	Crystal Violet	100 ml
-	PL.8000/4.0	Crystal Violet	400 ml
-	PL.8000/5.0	Crystal Violet	500 ml
-	PL.8001	Grams Iodine	100 ml
-	PL.8001/4.0	Grams Iodine	400 ml
-	PL.8001/5.0	Grams Iodine	500 ml
-	PL.8002	Neutral Red	100 ml
-	PL.8002/4.0	Neutral Red	400 ml
-	PL.8002/5.0	Neutral Red	500 ml
-	PL.8003	Safranin	100 ml
-	PL.8003/4.0	Safranin	400 ml
-	PL.8003/5.0	Safranin	500 ml
-	PL.8004	Dilute Carbol Fuchsin	100 ml
-	PL.8004/4.0	Dilute Carbol Fuchsin	400 ml
-	PL.8004/5.0	Dilute Carbol Fuchsin	500 ml
-	PL.8010	Lugols Iodine	100 ml
-	PL.8010/4.0	Lugols Iodine	400 ml
-	PL.8010/5.0	Lugols Iodine	500 ml
-	PL.8011	Methyl Violet	100 ml
-	PL.8011/4.0	Methyl Violet	400 ml
-	PL.8011/5.0	Methyl Violet	500 ml

### Per 100ml solution:

- Concentrated Crystal Violet contains 5g of Crystal Violet powder.
- Concentrated Grams Iodine contains 3.75g of Iodine.
- Concentrated Neutral Red contains 4g of Neutral Red powder.
- Concentrated Safranin contains 4g of Safranin O.
- Concentrated Dilute Carbol Fuchsin contains 1.5g of Basic Fuchsin powder.
- Concentrated Lugols Iodine contains 7.5g of Iodine.
- Concentrated Methyl Violet contains 5g of Crystal Violet powder.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loop
- Microscope
- Immersion Oil PL.396
- Pro-Slide™ Gram Stain Control PL.4961

### STABILITY AND STORAGE

The stains and differentiators should be stored at 15-25°C in their original containers. Product stored under these conditions will be stable until the expiry date shown on the product label.

### PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only. Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of staining.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.



- Samples should be processed in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

### TEST PROCEDURE

1. Prepare a smear on a clean glass slide and allow to air dry. Heat fix and allow to cool.
2. Flood the slide with Crystal Violet or Methyl Violet, stand for 1 minute. Rinse with water.
3. Flood the slide with Grams or Lugols Iodine, stand for 1 minute. Rinse with water.
4. Gently decolourise with differentiator for approx. 10 seconds or Iodine Acetone for 1 minute. Rinse with water.
5. Flood the slide with counterstain, stand for 30 – 60 seconds.
6. Rinse well with water; gently blot dry.
7. Examine using a microscope.

### QUALITY CONTROL PROCEDURE

Internal quality control of the stains and differentiators must be performed regularly on known reference material.

Recommended quality control:

Positive control – *Staphylococcus aureus* NCTC® 12981/ATCC® 25923\* (PLD13)  
 Negative control- *Escherichia coli* NCTC® 12241/ATCC® 25922\* (PLD02)  
 Pro-Slide™ Gram Stain Control PL.4961

### INTERPRETATION OF RESULTS

Gram positive organisms – blue to purple.  
 Gram negative organisms – pink to red.

### LIMITATIONS OF THE PROCEDURE

- Only experienced personnel should carry out the interpretation of stained slides
- Read prepared slides as soon as possible after staining. Failure to do so may affect the results.

### REFERENCES

- Anderson, N.L. et al. Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory. Coordinating ed., A.S. Weissfeld. *American Society for Microbiology*, Washington, D.C.
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- Chapin, K. C., and T.-L. Lauderdale. (2003). Reagents, stains, and media: bacteriology. p. 354-383.
- Gurr, E. (1953) A Practical Manual of Medical and Biological Staining Technique.
- Isenberg HD. (1992). Ed. Clinical microbiology procedures handbook, Vol I Washington, DC: *ASM Press*.
- Jorgensen et al. (1974) Manual of Clinical Microbiology. *American Society for Microbiology*, Washington, D.C.
- Public Health England. (May 2019). UK Standards for Microbiology Investigations: Staining Procedures. *Bacteriology – Test Procedures*. TP 39, Issue no.3.
- Lowrance, B.L., Reich, P. and Traub, W.H. (1969). *Journal of Applied Microbiology* 17:923-924.
- Murray, P. R., Baron, E. J., Jorgensen, J. H., Tenover, M. A. and Tenover, R. H. (ed.). (2003). Manual of clinical microbiology, 8th edition. *ASM Press*, Washington, DC.
- Wacko, R. and Sherris, J.C. (1963). *American Journal of Clinical Pathology*. 39:429-432.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use



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### HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

	PL.8000 PL.8000/4.0 PL.8000/5.0 PL.8011 PL.8011/4.0 PL.8011/5.0	H226, H318, H332, H350, H371, H411  P201, P210, P260, P273, P280, P305+P351+P338, P310, P370+P378, P391, P403+P235
	PL.7006/100 PL.7006/25 PL.7006 PL.7007 PL.7008	H226, H319, H331, H336, EUH066  P210, P261, P304+P340, P321, P370+P378, P403+P233

	PL.8001 PL.8001/4.0 PL.8001/5.0	H226, H315, H319, H373  P210, P260, P264, P321, P370+P378, P403+P235
	PL.8002 PL.8002/4.0 PL.8002/5.0	H226, H319, H332, H341  P210, P261, P280, P312, P370+P378, P403+P235
	PL.8010 PL.8010/4.0 PL.8010/5.0	H226, H302, H315, H319, H332, H373  P210, P260, P264, P370+P378, P403+P235, P501
	PL.7073 PL.7074 PL.7075	H226, H319, H350, H412  P201, P210, P280, P308+P313, P370+P378, P403+P235, P305+P351+P338, P337+P313
	PL.7306/25 PL.7306 PL.7307 PL.7308 PL.7406 PL.7407 PL.7408	H225, H302, H311, H319, H331, H371  P210, P260, P280, P321, P370+P378, P403+P233
	PL.8003 PL.8003/4.0 PL.8003/5.0	H226, H318, H332  P210, P261, P305+P351+P338, P310, P370+P378, P403+P235
	PL.8004 PL.8004/4.0 PL.8004/5.0	H302, H314, H332, H341, H410  P260, P273, P280, P303+P361+P353, P305+P351+P338, P310, P321, P391
	PL.7110 PL.7111 PL.7112	This mixture is classified as not hazardous according to regulation (EC) 1272/2008 [CLP].  Not classified (SI 2020/1567 as amended).



 <b>DANGER</b>	PL.7056 PL.7057 PL.7058 PL.7106 PL.7107 PL.7108 PL.7206/25 PL.7206 PL.7207 PL.7208	H225, H319, H336, EUH066  P210, P261, P312, P370+P378, P403+P233, P501
 <b>WARNING</b>	PL.7015/100 PL.7015/25 PL.7015 PL.7016 PL.7017	H226, H319, H400  P210, P273, P337+P313, P370+P378, P391, P403+P235
 <b>WARNING</b>	PL.7009/100 PL.7009/25 PL.7009 PL.7010 PL.7011 PL.7012/100 PL.7012/25 PL.7012 PL.7013 PL.7014 PL.7116 PL.7117 PL.7118	H226, H319  P210, P233, P337+P313, P363, P370+P378, P403+P235
 <b>DANGER</b>	PL.7000/100 PL.7000/25 PL.7000 PL.7001 PL.7002 PL.7113 PL.7114 PL.7115	H350, H412  P201, P202, P273, P280, P308+P313, P501
	PL.7100/CV PL.7101/CV PL.7102/CV	H350 P201, P202, P280, P308+P313, P405, P501
	PL.7003/100 PL.7003/25 PL.7003 PL.7004 PL.7005 PL.7052 PL.7053 PL.7053-2 PL.7101 PL.7102 PL.7103	EUH210  This mixture is classified as not hazardous according to regulation (EC) 1272/2008 [CLP].  Not classified (SI 2020/1567 as amended).