Gram Stains

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: see materials provided

CE

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.

PRINCIPLE

Gram postitve 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 leaving the final stain the chance to counterstain the bacteria leaving a 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	Gram's Iodine	100 ml
PL.7003/25	Gram's Iodine	250 ml
PL.7003	Gram's Iodine	500 ml
PL.7004	Gram's Iodine	1000 ml
PL.7005	Gram's Iodine	2000 ml
PL.7006/100	Gram's Differentiator	100 ml
PL.7006/25	Gram's Differentiator	250 ml
PL.7006	Gram's Differentiator	500 ml
PL.7007	Gram's Differentiator	1000 ml
PL.7008	Gram's 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	1000ml
PL.7017	Dilute Carbol Fuchsin	2000 ml
PL.7052	Lugol's Iodine	500 ml
PL.7053	Lugol's Iodine	1000 ml
PL.7053-2	Lugol's Iodine	2000 ml
PL.7056	Iodine Acetone	500 ml
PL.7057	Iodine Acetone	1000 ml
PL.7058	Iodine Acetone	2000 ml
PL.7100/CV	Crystal Violet 0.15%	500 ml
PL.7101	Basic Fuchsin / Neutral Red	500 ml
PL.7101/CV	Crystal Violet 0.15%	1000 ml
PL.7102	Basic Fuchsin / Neutral Red	1000 ml
PL.7102/CV	Crystal Violet 0.15%	2000 ml
PL.7103	Basic Fuchsin / Neutral Red	2000ml
PL.7073	C.Violet - Ammonium Oxalate	500 ml
PL.7074	C.Violet - Ammonium Oxalate	1000 ml
PL.7075	C.Violet - Ammonium Oxalate	2000 ml
PL.7110	Sandifords Stain	500 ml
PL.7111	Sandifords Stain	1000 ml
PL.7112	Sandifords Stain	2000 ml
PL.7113	Methyl Violet	500 ml
PL.7114	Methyl Violet	1000 ml
PL.7115	Methyl Violet	2000 ml
PL.7116	Safranin / Neutral Red	500 ml
PL.7117	Safranin / Neutral Red	1000 ml
PL.7118	Safranin / Neutral Red	2000 ml
PL.7206/25	Grams Differentiator (Acetone)	250 ml
PL.7206	Grams Differentiator (Acetone)	500 ml
PL.7207	Grams Differentiator (Acetone)	1000 ml
PL.7208	Grams Differentiator (Acetone)	2000 ml
PL.7306/25	Grams Differentiator (IMS)	250 ml
PL.7306	Grams Differentiator (IMS)	500 ml
PL.7307	Grams Differentiator (IMS)	1000 ml
PL.7308	Grams Differentiator (IMS)	2000 ml
PL.7406	Grams Differentiator	500 ml
PL.7407	Grams Differentiator	1000 ml
PL.7408	Grams Differentiator	2000 ml

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 deionized 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	Gram's Iodine	100 ml
PL.8001/4.0	Gram's Iodine	400 ml
PL.8001/5.0	Gram's 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	Lugol's Iodine	100 ml
PL.8010/4.0	Lugol's Iodine	400 ml
PL.8010/5.0	Lugol's 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

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loop
- Microscope
- Immersion oil PL.396
- Gram QC slides PL.4961

STABILITY AND STORAGE

Gram stains 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.

PROCEDURE

1. Prepare a smear on a clean glass slide and allow to 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 or iodine acetone for 1 minute. Rinse with water.
6. Flood the slide with counterstain, stand for 30 – 60 seconds.
7. Rinse well with water, gently blot dry.
8. Examine using a microscope.

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Canada 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3

U.S.A. (512) 832 9145 Fax (512) 832 6424
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046

UK 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL

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QUALITY CONTROL

Internal quality control of the Gram's stains must be performed regularly on known reference material.

Recommended quality control:

Positive control – *Staphylococcus aureus* NCTC®12981/ATCC®25923* (PLD.13)
Negative control- *Escherichia coli* NCTC®12241/ATCC®25922* (PLD.02)
CE marked Quality Control slides – PL.4961

INTERPRETATION OF RESULTS

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

LIMITATIONS

- 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

Available upon request.

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

EC REP

Advena Ltd. Tower Business Centre, 2nd Floor,
Tower Street, Swatar, BKR 4013, Malta.
T: +44(0) 1926 800 153

*NCTC® and NCPF® are trademarks of Public Health England. ATCC® strains are listed for reference only. ATCC® is a registered trademark of the American Type Culture Collection.

HAZARD IDENTIFICATION

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

 DANGER	PL.7000/100 PL.7000/25 PL.7000 PL.7001 PL.7002 PL.7100/CV PL.7101/CV PL.7102/CV PL.7113 PL.7114 PL.7115	H350, H412 P201, P202, P273, P280, P308+P313, P405, P501
 WARNING	PL.8001/4.0 PL.8001/5.0	H226, H315, H319, H373 P210, P280, P303+P361+P353, P305+P351+P338, P314, P501
	PL.8002 PL.8002/4.0 PL.8002/5.0	H226, H319, H341 P210, P280, P303+P361+P353, P305+P351+P338, P308+P313, P501
	PL.8010 PL.8010/4.0 PL.8010/5.0	H226, H315, H319, H373 P210, P264, P280, P305+P351+P338, P314, P501
 DANGER	PL.8000 PL.8000/4.0 PL.8000/5.0	H226, H318, H350, H411 P210, P270, P273, P280, P303+P361+P353, P304+P340, P310, P305+P351+P335, P501
	PL.8011 PL.8011/4.0 PL.8011/5.0	H226, H318, H350, H411 P210, P273, P280, P303+P361+P353, P305+P351+P338, P310, 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 PL.7110 PL.7111 PL.7112	Classification (EC 1272/2008) NC Not Classified.
 DANGER	PL.8003 PL.8003/4.0 PL.8003/5.0	H226, H318 P210, P280, P305+P351+P338, P310, P403+P235, P501

 DANGER	PL.7073 PL.7074 PL.7075	H226, H319, H350, H412 P201, P210, P273, P280, P308+P313, P303+P361+P353, P305+P351+P338, P501
	PL.7406 PL.7407 PL.7408	H225, H319, H332, H371 P210, P280, P303+P361+P353, P312, P304+P340, P305+P351+P338, P501
	PL.7306/25 PL.7306 PL.7307 PL.7308	H225, H319, H332, H371 P210, P270, P280, P303+P361+P353, P304+P340, P312, P501
 DANGER	PL.7006/100 PL.7006/25 PL.7006 PL.7007 PL.7008 PL.7056 PL.7057 PL.7058	H225, H319, H336 P210, P280, P303+P361+P353, P305+P351+P338, P312, P501
	PL.7206/25 PL.7206 PL.7207 PL.7208	H225, H319, H336 P210, P280, P303+P361+P353, P305+P351+P338, P312, P501
 WARNING	PL.7012/100 PL.7012/25 PL.7012 PL.7013 PL.7014	H226, H319 P210, P280, P303+P361+P353, P305+P351+P338, P337+P313, P501
	PL.7009/100 PL.7009/25 PL.7009 PL.7010 PL.7011	H226, H319 P210, P240, P305+P351+P338, P337+P313, P403+P235, P501
	PL.7015/100 PL.7015/25 PL.7015 PL.7016 PL.7017	H226, H319 P210, P280, P305+P351+P338, P337+P313, P403+P235, P501
	PL.7116 PL.7117 PL.7118	H226, H319 P210, P280, P305+P351+P338, P337+P313, P501
 DANGER	PL.8004 PL.8004/4.0 PL.8004/5.0	H226, H302, H314, H341, H351, H412 P210, P273, P280, P301+P330+P331, P304+P340, P303+P361+P338, P501

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UK 0151 353 1613 Fax 0151 353 1614
3 Bassendale Road, Bromborough, Wirral, Merseyside, CH62 3QL

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Canada 800 268 2341 Fax 905 731 0206
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3

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