Cryptosporidium Staining

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: SEE MATERIALS PROVIDED

CE

INTENDED USE

For use in detecting oocysts of *Cryptosporidium* species in prepared faecal smears from clinical specimens.

SUMMARY AND EXPLANATION

Cryptosporidium was first identified in 1976 and is one of the most common waterborne diseases found worldwide. Modifications of the acid-fast staining procedure can be used to identify *Cryptosporidium*; this method uses a high concentration of phenol to facilitate penetration of the basic fuchsin dye into the cell wall of oocysts.

PRINCIPLE OF THE TEST

Cell wall components of *Cryptosporia* oocysts form a complex with Carbol Fuchsin which is retained in the cell wall after decolourisation. A counterstain is then used to emphasise the stained oocysts.

MATERIALS PROVIDED

Ready to use Stains and Differentiators:

-	PL.7072	Cryptosporidium Fixative	500 ml
-	PL.7062	Cryptosporidium Stain	500 ml
-	PL.7065	Differentiator 1	500 ml
-	PL.7068	Differentiator 2	500 ml
-	PL.7071	Cryptosporidium Counterstain	500 ml

Per 100ml solution:

- Cryptosporidium Stain contains 2.95g of Basic Fuchsin powder and 15.63 g of Phenol.
- Differentiator 1 contains 1ml of Hydrochloric Acid and 99ml of IMS.
- Differentiator 2 contains 1ml of Acetic Acid and 99ml of IMS.
- Cryptosporidium Counterstain contains 0.4g of Malachite Green powder.

Staining Kits (ready to use):

- PL.8060 Cryptosporidium Staining Kit
  1 x PL.7072, 1 x PL.7062, 2 x PL.7065, 2 x PL.7068, 1 x PL.7071

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loops
- Microscope
- Immersion Oil PL.396
- Pro-Slide™ Cryptosporidium Stain Control PL.4962

STABILITY AND STORAGE

The stains and differentiators should be stored at 15°C-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 the 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 smear by emulsifying in saline on a clean glass slide. Allow to air dry.
2. Place slide on a staining rack and fix in Cryptosporidium Fixative for 1 minute. Allow to air dry.
3. Flood the slide with Cryptosporidium Stain, stand for 5 minutes.
4. Pour off excess stain and decolourise with Differentiator 1 until no more stain washes out of the smear. Rinse with water and shake off any excess.
5. Decolourise with Differentiator 2 for 2 minutes. Rinse with water and shake off any excess.
6. Flood the slide with Cryptosporidium Counterstain, stand for 1 minute.
7. Rinse well with water and gently blot dry, or dry using gentle heat.
8. Examine using a microscope.

N.B. PL.7076 Cryptosporidium Differentiator (500ml) can be used as an alternative to Differentiator 1 and Differentiator 2.
To use Cryptosporidium Differentiator, steps 4 and 5 of the Test Procedure should be replaced with a single step: 'Decolourise with Cryptosporidium Differentiator for 2 minutes or until no more stain washes out of the smear.'
PL.7076 is sold separately, and not as part of the Cryptosporidium Staining Kit (PL.8060).

- Cryptosporidium Differentiator contains 1ml of Hydrochloric Acid and 99ml of Methanol.

QUALITY CONTROL PROCEDURE

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

Recommended quality control:
Positive control – A proven positive
Negative control – A proven negative
Pro-Slide™ Cryptosporidium Stain Control PL.4962

INTERPRETATION OF RESULTS

Cryptosporidia oocysts are stained bright pink-red. Decolourised background material will appear as pale green or pale red in colour.

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.
- False staining results can be seen due to cellular debris being stained by the technique.
- Organisms other than *Cryptosporidium* species may display varying degrees of acid fastness, e.g. *Rhodococcus* spp., *Mycobacteria* spp., and *Isospora* spp.

REFERENCES

- Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R.H.A. The Practice of Medical Microbiology. 12th Edition. V2
- Kinyoun, J.J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. *American Journal of Clinical Pathology*. 46:472-4.
- Lennette. 1974. Manual of Clinical Microbiology. *American Society for Microbiology*, Washington, D.C.
- Neelson, F. 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. *Centraldl. Med. Wiss.* 21:497-501.
- Public Health England. May 2019. UK Standards for Microbiology Investigations: Staining Procedures. *Bacteriology – Test Procedures.* TP 39, Issue no.3.
- Ziehl, F. 1882. Zur Farbung des Tuberkelbacillus. *Dtsch. Med. Wochenschr*. 8:451.

	= 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
i	= Consult instructions for use

EC REP Advena Ltd. Tower Business Centre, 2nd Floor, Tower Street, Swatar, BKR 4013, Malta.

Canada: Toll Free +1 800 268 2341 Tel +1 905 731 0300
20 Mural Street, Unit 4, Richmond Hill, ON, L4B 1K3
www.pro-lab.com

U.S.A: Tel (512) 832-9145
21 Cypress Blvd, Suite 1155, Round Rock, Texas, 78665-1046
www.pro-lab-direct.com

UK: Tel +44 (0)151 353 1613
3 Bassendale Road, Bromborough, Wirral, CH62 3QL
www.pro-lab.co.uk

Revision: 2025 01

HAZARDS IDENTIFICATION

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

DANGER	PL.7072	H225, H301, H311, H331, H336, H370 P210, P260, P301+P310, P321, P370+P378, P403+P233
	PL.7076	H225, H301, H311, H331, H370 P210, P260, P301+P310, P321, P370+P378, P403+P233
	PL.7065 PL.7068	H225, H302, H311, H319, H331, H371 P210, P260, P280, P321, P370+P378, P403+P233
DANGER	PL.7062	H302, H312, H314, H332, H341, H373, H410 P260, P273, P280, P303+P361+P353, P305+P351+P338, P310, P321, P391
WARNING	PL.7071	H226, H319, H412 P210, P273, P337+P313, P370+P378, P403+P235, P501