

1. Introducing *EasyStain*™

EasyStain™ is an immunofluorescence reagent designed for use in testing water samples for the presence of *Cryptosporidium* oocysts and *Giardia* cysts. When a water sample is stained with the *EasyStain*™ reagent all oocysts or cysts present in the sample will fluoresce bright green. Stained samples can then be examined using fluorescence microscopy¹, flow cytometry² or laser scanning cytometry³.

The detection of oocysts and cysts in water is extremely difficult. There are many particles present in water samples that cross-react with *Cryptosporidium* and *Giardia*-specific antibodies. These particles can easily be mistaken for *Cryptosporidium* oocysts or *Giardia* cysts⁴. Unlike other *Cryptosporidium* and *Giardia* antibodies, *EasyStain*™ does not cross-react with particles present in water samples. This superior specificity is achieved by using monoclonal antibodies (mAbs) of the IgG1 isotype⁵.

Until now, all commercially available *Cryptosporidium*-specific and *Giardia*-specific mAbs were IgM or IgG3 isotype antibodies⁵, which bind to algae, mineral particles, bacteria, yeast and many other particles.

EasyStain™ utilises a patented method for producing IgG1 antibodies to *Cryptosporidium* and *Giardia*. These antibodies have been demonstrated to be far superior to other available antibodies^{5,6}.

The antibodies in the *EasyStain*™ have been purified to a very high level to ensure that there is no contamination. The conjugated antibody is stored in a specially designed buffer that includes a novel blocking agent and a preservative.

2. Product description

EasyStain™ is a mixture of two monoclonal antibodies (mAb): a *Cryptosporidium*-specific IgG1 mAb and a *Giardia*-specific IgG1 mAb. The reagent is supplied as a working solution and does not require dilution.

A Wash Buffer is supplied that is used to wash excess antibody from slides or membranes. The Wash Buffer has been specially designed to increase fluorescence and reduce fading. It does not require dilution.

A Positive Control, containing both irradiated *Cryptosporidium* oocysts and *Giardia* cysts is supplied. Cysts and oocysts are at a concentration of approximately 200 to 400 per 50 µl.

Mounting Medium is supplied that is applied to the microscope slide before adding a coverslip. The Mounting Medium has been specially designed to reduce fading of the prepared sample.

3. Quality Control

Two forms of quality control are performed on *EasyStain*™:

1. The mean fluorescence intensities (MFI) of oocysts and cysts stained with *EasyStain*™.
2. Quality control of the non-specific binding properties of the antibody.

EasyStain™ is supplied with a Certificate of Analysis.

Each batch of *EasyStain*™ must meet the following standards:

Mean fluorescent intensity of <i>Giardia</i> (A)	> 700
Mean fluorescent intensity of <i>Cryptosporidium</i> (A)	> 500

A) Measured using flow cytometry
B) Number of *Cryptosporidium* and *Giardia* like particles detected by flow cytometric analysis of a standardised water sample stained with the antibody⁸.

4. Materials required but not supplied

- Glass Teflon coated well slides
- Pipettes
- Coverslips
- DAPI:(diamino4',6-diamidino-2-phenylindole dihydrochloride hydrate) - Sigma Chemical Co. Ltd
- Dissolve in methanol at a concentration of 2mg/ml
- This stock is stable at 4°C for 4 months
- To prepare a working solution dilute 1 in 1000 in PBS. The working solution is stable at 4°C for 2 weeks.

If staining is to be performed on a membrane rather than a microscope slide then the following materials are required:

- Membranes; 13mm diameter, 0.8µm pore-size – Millipore ATTP01300 or equivalent.
- Flat-bladed forceps
- Either a Membrane Manifold and a vacuum source, OR
- A vacuum source with a liquid trap connected to a 13mm Swinnex filtration unit (Millipore SX0001300).

5. Instructions for use

A. Using *EasyStain*™ to stain a water sample on a membrane

The following method describes how to stain a water sample on a 13mm diameter membrane.

1. Apply a vacuum to the membrane (<-0.4 bar) and carefully pipette the sample onto the surface of the membrane.
2. Rinse the membrane with 1ml of distilled water.
3. Add 50µl of DAPI stock (2mg/ml) into 50ml PBS to make a working strength solution of DAPI.
4. Turn off the vacuum and add 100µl of the working strength DAPI solution and leave for 2 minutes.
5. Turn on the vacuum to remove the DAPI.
6. Turn off the vacuum and apply 100µl distilled water. Leave for 1 minute.
7. Turn on the vacuum to remove the water.

8. Turn off the vacuum and apply 100µl of *EasyStain*TM (Antibody solution).
9. Incubate at room temperature for 30 minutes or at 37°C in a humidified chamber for 15 minutes.
10. Turn on the vacuum to remove the antibody.
11. Turn off the vacuum and apply 120µl of Wash Buffer to the surface of the membrane. Leave for 1 minute.
12. Turn on the vacuum and rinse the membrane by slowly applying 1ml of Wash Buffer to the surface of the membrane.
13. Turn off the vacuum.
14. Pipette 3µl of Mounting Medium onto a microscope slide.
15. Carefully pick up the membrane with forceps and lay it onto the Mounting Medium.
16. If a coverslip is required apply Mounting Medium to the coverslip before placing over the membrane.

B. Using *EasyStain*TM and DAPI to stain a water sample on a microscope slide.

Optimized procedure for staining cysts and oocysts with both *EasyStain*TM and DAPI.

1. Place the sample onto a well slide and allow to dry at a temperature of no greater than 37°C.
2. Add 50µl methanol to the well and allow to dry at room temperature.
3. Add 50µl of DAPI stock (2mg/ml) into 50ml PBS to make a working strength solution of DAPI.
4. Add 50µl of the working strength DAPI solution and leave for 2 minutes.

5. Carefully tip the slide, long edge down to aid removal of the DAPI. Use a Pasteur pipette to carefully remove the DAPI. Ensure that you do not touch the surface of the well slide with the Pasteur pipette.
6. Add 50µl of distilled water to the well and allow to stand for 1 minute.
7. Carefully tip the slide, long edge down to aid removal of the distilled water. Use a Pasteur pipette to carefully remove the distilled water. Ensure that you do not touch the surface of the well slide with the Pasteur pipette.
8. Add 50µl *EasyStain*TM (Antibody solution) to the well and at room temperature for 30 minutes or at 37°C in a humidified chamber for 15 minutes.
9. Carefully tip the slide, long edge down to aid removal of antibody. Use a pasteur pipette to carefully remove the antibody. Ensure that you do not touch the surface of the well slide with the Pasteur pipette
10. Slowly add 300µl of Wash Buffer to the well and allow to stand at room temperature for 1 minute. The Wash Buffer should flow over the edges of the well slide. This will help to reduce background staining around the edges of the slide.
11. Gently aspirate the excess Wash Buffer from below the well using a clean pasteur pipette. Whilst aspirating the Wash Buffer, carefully tip the slide, long edge down to aid removal of the buffer.
12. Add 2-3µl of Mounting Medium to the well and apply a coverslip.
13. Record the date and time that staining was completed on the bench sheet. Slides may be stored in a humid chamber in the dark at 2°C to 8°C until ready for examination.

6. Notes

If a sample contains a high concentration of salt, salt crystals formed after drying can cause cysts and oocysts to wash off the slide and may interfere with staining. The sample should be washed in distilled water before applying to the slide. Alternatively, the sample may be stained on a membrane.

The Wash Buffer has been specially formulated to minimise background and fading. It is essential that the Wash Buffer is applied to the stained cysts and oocysts for a minimum of 1 minute.

The Mounting Medium has been specially formulated for use with IgG1 antibodies. The use of alternative Mounting Mediums may result in fading of oocysts and cysts.

7. References

1. Standard Operating Protocols for the Monitoring of *Cryptosporidium* Oocysts in Treated Water Supplies to Satisfy Water Supply (Water Quality) Amendment Regulations 2000 SI No 3184. UK Drinking Water Inspectorate.
2. Vesey, G, Slade, JS, Byrne, M, Shepherd, K, Dennis, P.J.L, and Fricker, CR. 1993. Routine monitoring of *Cryptosporidium* oocysts in water using flow cytometry. Journal of Applied Bacteriology, 75, 82-86, 1993.
3. Zanelli F, Compagnon B, Joret JC, de Roubin MR. Enumeration of *Cryptosporidium* oocysts from surface water concentrates by laser-scanning cytometry. Water Science & Technology. 41(7):197-202, 2000.
4. Clancy J.L. Gollnitz W.D. Tabib Z. Commercial labs - how accurate are they? Journal American Water Works Association. 86(5):89-97, 1994.
5. Weir C, Vesey G, Slade M, Ferrari B, Veal D, William K. An immunoglobulin G1 monoclonal antibody highly specific to the wall of *Cryptosporidium* oocysts, Clinical and Diagnostic Laboratory Immunology, 7(5): 745-750, 2000.
6. Ferrari BC, Vesey G, Weir C, Williams KL, Veal DA. Comparison of

Cryptosporidium-specific and *Giardia*-specific monoclonal antibodies for monitoring water samples. Water research, 33(7): 1611-1617, 1999.

7. Haugland, R.P. Coupling of monoclonal antibodies with fluorophores. In Methods in Molecular Biology, Vol. 45: Monoclonal Antibody Protocols ed. Davis, W.C.pp. 205-221. Totowa, N.J., USA: Davis Humana Press Inc. 1995.
8. Vesey, G, Deere, D, Weir, C, Ashbolt, N, Williams, K, and D. Veal. A method for evaluating *Cryptosporidium*-specific antibodies for testing water samples. Letters in Applied Microbiology, 25, Issue 5, <NOBR> 316-320, 1997.

Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the product labels for the duration of the stated shelf life. BTF's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at BTF's expense, of any products which shall be defective in manufacture, and which shall be returned to BTF, transportation prepaid, or at BTF's option, refund of the purchase price. Claims for merchandise damaged in transit must be submitted to the carrier. This warranty shall not apply to any products which shall have been altered outside BTF, nor shall it apply to any products which have been subjected to misuse or mishandling. ALL OTHER WARRANTIES, EXPRESSED, IMPLIED OR STATUTORY, ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. BTF's maximum liability is limited in all events to the price of the products sold by BTF. IN NO EVENT SHALL BTF BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow limits on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply.

©Copyright 2001 BTF Pty. Limited, Sydney, Australia.

Issue: 5
Date: 22nd July 2003

BTF Pty Ltd

PO Box 599 North Ryde BC NSW 1670 Australia
Telephone +61 2 8877 9150 Facsimile +61 2 8877 9101
contact@biotechfrontiers.com <http://www.biotechfrontiers.com>

