EasyStain™

Instructions for Use

Optimized for USEPA Method 1623

1. Place the IMS-separated sample onto a well slide and neutralize according to IMS suppliers instructions.

2. (a) If staining immediately, dry at 37°C (maximum drying time 1 hour). Immediately perform steps 3 to 12.

   (b) If there will be a delay in staining:

   (i) Dry at 37°C (maximum drying time 1 hour). Place in a refrigerator at 4°C.

   (ii) Alternatively, dry the slide (uncovered) overnight in a refrigerator at 4°C.

Tip: Do not dry slides in a cool room as some cool rooms maintain a humid environment.

3. This step can improve DAPI staining and is optional. Add 50 µl methanol to the well. Allow to dry (typically 30 minutes) and follow step 4 immediately.

4. Add 1 – 2 drops of EasyStain™ DAPI *(or 50µl working strength DAPI) solution to the well. Leave on for 2 minutes.

5. Tilt well slide (long edge down). Using a Pasteur pipette, gently remove excess EasyStain™DAPI from below the well. Alternatively, use absorbent material (placed at edge of well slide)

Tip: Do not touch well surface with pipette tip or absorbent material, as this will disturb the sample.

6. Add 50 µl distilled water to the well. Leave for 1 minute.

7. Tilt well slide (long edge down). Using a Pasteur pipette, gently remove excess water from below the well. Alternatively, use absorbent material (placed at edge of well slide).

8. Add 50 µl EasyStain™ to the well. Incubate at room temperature for 30 minutes or in a box containing moist tissue at 37°C for 15 minutes.

9. Tilt well slide (long edge down). Using a Pasteur pipette, gently remove excess EasyStain™ from below the well. Alternatively, use absorbent material (placed at edge of well slide).

10. Slowly add 300 µl ice cold Fixing Buffer to the well and allow to flow over well edges. Leave for 2 minutes. Critical: The Fixing Buffer must be ice cold and left for 2 minutes.

NOTE: If you wish to perform genotyping on oocycts recovered from slides, replace the 300 µl of fixing buffer with 300 µl of PBS. (The PBS may be at room temperature)**

11. Tilt well slide (long edge down). Using a Pasteur pipette, gently remove excess Fixing Buffer from below the well. Alternatively, use absorbent material (placed at edge of well slide).

12. Add 5 µl EasyStain™ Mounting Medium to the well. Apply cover slip. Alternatively, add 5 µl EasyStain™ Mounting Medium to the center of a cover slip (placed on the bench). Hold well slide on its edge (long edge down) next to cover slip. Gently lower well slide (sample down) onto cover slip. Critical: Only use the EasyStain™Mounting Medium supplied in the kit. Other Mounting Mediums will cause fading.

*Note: Steps 6 & 7 maybe omitted at the discretion of the Laboratory.
Step 10: Adjust volume of FB to the size of the well e.g. 9mm well use 100ul FB.

Important

- Use Fixing Buffer directly from the refrigerator or keep on ice while using.
- Steps 8 to 11 can be completed before steps 4 to 7 (DAPI can follow EasyStain™).

*Working strength DAPI solution is 50 µl DAPI stock (2 mg DAPI dissolved in 1 ml methanol) added to 50 ml PBS. Make up the working strength DAPI solution daily

**Di Giovanni, G. D., R. M. Hoffman, and G. D. Sturbaum. 2010. Cryptosporidium Genotyping Method for Regulatory Microscope Slides, Project 4099 Report. Denver, CO: Water Research Foundation. http://www.waterrf.org/Pages/Projects.aspx?PID=4099

Technical assistance

For technical assistance with the use of EasyStain™ or other *Cryptosporidium* and *Giardia* testing issues please email info@btfbio.com or Fax: +61 2 8877 9101.

Certificates of Analysis

Certificates of Analysis can be downloaded from the BTF website: www.btfbio.com.