IMS400 Crypto-Grab™ IMS Beads: IMS Beads for Separation and Isolation of *Cryptosporidium spp. Oocysts* from Water Particulates

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Introduction: *Cryptosporidium spp.* is a common, ubiquitous intestinal parasitic protozoan that infects man and lower animals. This organism has a broad reservoir of host animals in the wild and can be spread through food, water, and fomites. The infectious oocyst stage is commonly found in particulates isolated from environmental samples, including surface water.

Description: The Crypto-Grab™ IMS beads provided in the Crypto-Grab™ IMS kit are designed for capture and isolation of *Cryptosporidium spp.* oocysts from complex mixtures of particulates isolated from surface or drinking water using U.S. EPA Method 1623. The beads are provided as a suspension of superparamagnetic particles that measure approximately 1.6 um in diameter and are coated with our own mouse monoclonal antibodies prepared specifically against antigens located on the outer surface of oocysts of these parasite.

Kit Includes:
- Crypto-Grab™ IMS Beads, 2 mL
- Grab™Buffer-A, 2X concentrated, 100 mL
- Grab™Buffer-B, 1X, 30 mL

Storage: Store kit at 4-8° C. Do not freeze. The storage solution consists of a phosphate-buffered saline (PBS) containing Tween 20, bovine serum albumin, EDTA, and 0.04% sodium azide as preservative.

Note: Kit does not include all supplies and equipment associated with sample purification by US EPA Method 1623, Section 13, December 2005 version.

Items Required for IMS Procedure:
- Prepared Sample in deionized water: Environmental Sample or QC Standard
- Crypto-Grab™ IMS Beads, 0.1 ml to be used per sample
- Grab™ Buffer-A at 2X concentration, 5 mL per sample – bring to room temperature
- Grab™ Buffer at 1X concentration, 1.5 mL per sample – bring to room temperature
- Flat-sided (Leighton) tube: 125 x 16 mm with 11 x 50 window (flat-side)
- Large magnetic particle concentrator, e.g. Dynal/Invitrogen MPC®-1 magnet
- Small magnetic particle concentrator, e.g. Dynal/Invitrogen MPC®-S, –M, magnets or DynaMag™-Spin magnet
- 1.5 mL microtubes
- Timer
- Vortex Mixer
- Rotating tube mixer (e.g. Dynal/Invitrogen 'Rotamix')

Other Lab Supplies Not Included, but Available:
- Waterborne™, Inc. fluorescent monoclonal antibody reagents (Please visit: www.waterborneinc.com)
- 1.0 mL positive control: 2x10/5 cells each *G. lamblia* and *C. parvum* in fixative.
- 50 mL 20x SureRinse™ Wash Buffer
- C101: 3.5 mL BlockOut™ Counterstain
- D101: 400 uL 5000x concentrated DAPI solution in methanol
- M101: 3.5 mL No-Fade™ Mounting Medium
- M102: 3.5 mL Elvanol No-Fade™ Mounting Medium
- S100-1-9MM: One-well SuperStick™ Slides, 9 mm diam. well, 40/box
- S100-1: One-well SuperStick™ Slides, 14 mm diam. well, 40/box
- S100-2: Two-well SuperStick™ Slides, 15 mm diam. wells, 40/box
- PAC-3, -6, or -12: Flow cytometrically prepared standards of *Cryptosporidium* and *Giardia* (AccuSpike™-IR product), 100 each per vial, preserved by gamma irradiation, EPA-validated

Differences from the currently published Method 1623: Note that certain steps below differ from the US EPA published Method 1623, version of Dec. 2005. **These differences are marked with an asterisk (*).**

Sample Preparation: Water particulates should be isolated by filtration of surface or drinking water, using filters and procedures described in US EPA Method 1623. The particulates, 0.5 mL volume packed pellet or less, should be suspended in a volume of **3 mL*** capsule filter eluate or deionized water. (If the packed pellet volume is greater than 0.5 mL, then it must be subdivided into sub-samples, as the Method states.)

Instructions for Use:
1. Allow sample, Crypto-Grab™ IMS particles, and all reagents to equilibrate to room temperature before proceeding with the IMS procedure. Samples must be in **3 mL*** deionized water or capsule filter eluate. If the sample is stored in any other solution than deionized water or capsule filter eluate from Method 1622 or 1623, it must be rinsed free of the solution, by centrifugation, and re-suspended in deionized water.
2. Use a graduated, 10-mL pipette that has been pre-rinsed with elution buffer to transfer the water sample to the flat-sided (Leighton) tube(s). Rinse the centrifuge tube twice with approx. 1 ml deionized water and add the rinsates to the flat-sided tube containing the sample. Sample volume will now be 5 mL.
3. To this tube, add **5 mL*** of the **2X** conc. Grab™ Buffer-A*. Buffer should be at room temperature. The volume in the flat-sided tube will be 10 mL at this point. Cap tube tightly and mix by inversion 3 times.
4. Suspend the Crypto-Grab™ beads in the vial in which they arrived by vortexing for approximately **20** seconds. Ensure visually that there is no unsuspended residuum at the bottom of the vial.
5. Transfer **100 uL** (0.1 mL) of the Crypto-Grab™ IMS bead suspension to the flat-sided tube containing the water particulates. Cap the tube tightly and proceed with the rotation step in Method 1623, section 13.3.2.6. (That is, rotate the sample/IMS bead suspension on a rotating mixer (18 rpm) at room temperature for **1 hour**.)
6. Remove the tube from the rotating mixer after **1 hour** and place the tube into a magnetic particle concentrator (e.g. Dynal/Invitrogen MPC®-1), flat-side toward the magnet.
7. While the tube is firmly attached to the magnet, turn the tube flat-side down (toward the table) and gently/slowly rock the tube and magnet end-to-end (cap to base) through approximately 90°, according to the Method, sections 13.3.2.9 – 13.3.2.10. After **3.0* minutes**, with the tube tightly held in magnet, return the magnet and tube to the upright position, uncap, pour off supernatant and discard. Allow the tube to rest, while still attached to the magnet, and use a Pasteur pipet to remove any residual liquid from the bottom of the tube. Do not disturb the beads.
8. Remove the tube from the magnet and add 0.48 mL* (480 ul) of Grab™ Buffer-B. Mix gently by rocking the tube to suspend the beads from the flat side of the tube. Gentle flushing of the flat area of the tube using a Pasteur pipet is also necessary. (See Method 1623 Supplemental Information of July 10, 2007 from EPA for updated advice on this.)
9. Using a Pasteur pipet, transfer the 0.48 mL volume of bead suspension to a 1.5 mL microtube. Rinse the flat-sided tube twice with 0.48 mL Grab™ Buffer-B, rocking the tube gently, and carefully flushing the flat side only with a Pasteur pipet. Using the same Pasteur pipet, add these rinses to the same 1.5 mL microtube. Try to avoid bubbling. Wait approx. 15 sec., then collect and transfer any remaining liquid from the flat-sided tube.
10. Proceed with the EPA Method, sections 13.3.2.14 to 13.3.2.16, by capping and placing the 1.5 mL microtube into the small (MPC-S) magnet, or SpinMag, and rocking it end-to-end (cap to base), through approximately 180°. Continue rocking for 1 minute.
11. While the tube is attached to the magnet, aspirate off and discard *all* of the supernatant with a Pasteur pipet, being careful not to disturb the pellet that has formed.
12. At this point, proceed with the oocyst-bead dissociation according to Method 1623, by removing the tube from the small magnet and adding **50 uL** aqueous 0.1 N HCl solution.
13. Cap the tube and vortex vigorously for 50 seconds to re-suspend the particles/oocysts.
14. Allow the tube to stand at room temperature for at least 10 minutes.
15. Vortex the microtube vigorously for 30 seconds.
16. Place the microtube into the small magnet for 30 seconds, then remove the 50 ul of supernatant using a micropipet (without disturbing the pellet that has formed) and transfer it to the well of a Waterborne™, Inc., SuperStick™ (or other appropriate slide) to which 8 ul* of 1.0 N NaOH solution has been applied, as described in the Method.
17. Repeat oocyst-bead dissociation (Steps 12 – 16). The samples may be combined or placed on two separate wells.
18. Air-dry the sample to complete dryness with a gentle stream of warm - but not hot - air.
19. Proceed to the fluorescent antibody and DAPI staining procedure as described in the Method.
20. View and count oocysts under fluorescence microscope.