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 infect 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 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 this 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, http://www.epa.gov/microbes/1623de05.pdf.

Items Required for IMS Procedure:
- Prepared Sample in deionized water: Environmental Sample or QC Standard
- Crypto-Grab™ IMS Beads, 0.1 ml (100 ul) to be used per sample; vortex 10 sec. to suspend
- 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 (e.g. Barnstead Maxi-Mix I or Vortex-Genie II (Scientific Products Inc.)
- Rotating tube mixer (e.g. Dynal/Invitrogen 'Rotamix')

Other Lab Supplies Not Included, but Available:
- AccuSpike™-IR product (PAC-3, -6, or –12): Flow cytometrically prepared standards of *Cryptosporidium* and *Giardia*, 100 each per vial, preserved by gamma irradiation, US EPA-validated
- Waterborne™, Inc. fluorescent monoclonal antibody reagents, all US EPA-validated (Please visit: www.waterborneinc.com)
- 50 mL 20x SureRinse™ Wash Buffer for IFA procedure
- C101: 3.5 mL BlockOut™ Counterstain
- D101: 400 uL 5000x concentrated DAPI solution in methanol
- M101: 3.5 mL No-Fade™ Mounting Medium
- M101FF: 3.5 mL Formalin-Free 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

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 (see link above). The particulates, 0.5 mL volume packed pellet or less, should be suspended in a volume of 5 mL capsule filter eluate or deionized water (preferably the latter). (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 5 mL deionized water or filter eluate. If the sample is stored in any other solution than deionized water or capsule filter eluate (Elution Buffer) 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 **Grab™ Buffer-A*** and add the 'rinsates' to the flat-sided tube containing the sample. Sample volume will now be 7 mL.
3. To this tube, add **3 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 capped vial by vortexing vigorously for approximately **10** 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 (approx. 20 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 **450 ul* of Grab™ Buffer-B**. Mix gently by rocking the tube horizontally to suspend the beads from the flat side of the tube. Gentle flushing of the flat area of the tube using a Pasteur pipet might be necessary. (See Method 1623 Supplemental Information of July 10, 2007 from EPA for updated advice on this.)
9. Using a Pasteur pipet, transfer the 450 ul volume of bead suspension to a 1.5 mL microtube. Rinse the flat-sided tube twice with 450 ul mL Grab™ Buffer-B, again rocking the tube gently. Using the *same* Pasteur pipet and very gentle pipetting, add these rinses to the same 1.5 mL microtube. Try to avoid bubbling. Wait approx. 10 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 and discard *all* of the supernatant with a Pasteur pipet, being very careful not to disturb the pellet of beads that has formed.
12. At this point, proceed with the oocyst-bead dissociation, beginning by removing the tube from the small magnet and adding **50 uL** aqueous 0.1 N HCl solution.

Dissociation: Note that our recommended vortexing times **differ** from those given in Method 1623. Excessive vortexing with Waterborne™ IMS beads is counterproductive and may result in reduced recovery. Medium-strong to strong vortexing intensity is best. Recommended vortexing times vary with the type/brand of vortex-mixer used. Two commonly used brands are the single-setting Barnstead Maxi-Mix I and the variable-setting Vortex-Genie II (Scientific Products Inc.). Vortexing times and wait periods for each of the above vortex mixers are listed below.

Barnstead Maxi-Mix I, Single Speed

- Vortex microtube for 30 seconds*; allow the tube to stand at room temperature for at least 10 minutes; vortex the microtube again for 30 seconds.
- Place the microtube onto the small magnet for a minimum of 20 seconds, then remove the 50 ul of supernatant using a micropipet (without disturbing the pellet of beads that has formed) and transfer it to the well of a Waterborne™, Inc., SuperStick™ (or other appropriate slide) to which 10 ul of 1.0 N NaOH solution has been applied.
- Repeat this procedure once with an additional 50 ul 0.1 N HCl. Add the second supernatant to the first in the same well, swirling the combined supernatants slightly with the micropipet tip. Note that the 10 uL droplet of 1 N NaOH, used here to neutralize the acid, is only added once, before transferring the first supernatant.

Vortex-Genie II, or other Variable Speed vortex-mixer

- Use as above-described with single-speed vortex-mixer, but us setting of approximately 8.5-9.0 out of a maximum of 10.

13. Air-dry the sample to complete dryness at room temperature, or using a gentle stream of warm - but not hot - air.
14. Proceed to the fluorescent antibody and DAPI staining procedure as described in the Method.
15. View and count oocysts under fluorescence microscope.