applied biosystems
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Dynabeads® GC-Combo

Catalog nos. 73002, 73012 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 015)

Kit Contents

Kit contents	73002	73012
Dynabeads® anti-Cryptosporidium	1 × 1 mL	1 × 5 mL
Dynabeads® anti-Giardia	1 × 1 mL	1 × 5 mL
10X SL™-Buffer A	1 × 15 mL	1 × 60 mL
10X SL™-Buffer B	1 × 15 mL	1 × 60 mL

Kit capacity

Capacity for 73002: 10 tests
Capacity for 73012: 50 tests

Dynabeads® anti-Cryptosporidium and Dynabeads® anti-Giardia are supplied as a suspension in phosphate buffered saline (PBS), pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide. For more information on the 10X SL-Buffer A and 10X SL-Buffer B, see "Description of Materials".
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Intended Use

Dynabeads® GC-Combo is designed for rapid, selective separation of *Giardia* cysts and *Cryptosporidium* oocysts from water sample concentrates. Immunomagnetic separation (IMS) using Dynabeads® anti-Cryptosporidium and Dynabeads® anti-Giardia is conducted in SL-Buffer. This is a two-component buffer system which has been specifically designed for efficient separation of both *Giardia* cysts and *Cryptosporidium* oocysts from a wide range of water types.

The antibodies coated on the beads selectively bind cysts and/or oocysts within the water sample concentrate and form a complex. The Dynabeads®-organism complexes are separated using an MPC™ magnet or an automated magnetic separation instrument (BeadRetriever™), and subsequently the cysts and oocysts are dissociated from the beads.

Intended User

Any scientist skilled, equipped, and/or certified for *Giardia* and *Cryptosporidium* testing on water and environmental samples may use Dynabeads® GC-Combo for IMS. The user must be skilled in using conventional microbiological techniques and interpreting results. Screening of slides for enumeration of separated oocysts/cysts must be performed by a user who is proficient in recognition of *Giardia* cysts and *Cryptosporidium* oocysts.

Sample Matrix

The water sample concentrates can be of any treated water or untreated (raw) water which is intended for potable supply. The water sample concentrates should be prepared by standard filtration and centrifugation methods (e.g. US EPA Method 1623 and UK Water Supply Regulations 2000).

- Use ≤10 mL water sample concentrate per tube.
- Centrifuge at 1050 × g for 10 min, remove the supernatant, and add demineralized oocyst free water so the final volume contains ≤0.5 mL packed pellet per 10 mL.
- If the final volume contains ≥0.5 mL packed pellet per 10 mL, then split the sample in two equal aliquots.
- Wash the seed cysts/oocysts twice in PBS/Tween®, and once in demineralized water before seeding the water sample.

Required Materials

- Magnets: MPC™-6, MPC™-1, MPC™-S.
- Mixer allowing tilting and rotation of tubes (e.g. MX 1, Sample Mixer).
- L-10 tubes, Spot-On.
- Filters, centrifuges, and other equipment for preparation of the water sample concentrate from the original water sample.
- Vortex mixer.
- Test-tubes, glassware, pasteur pipettes, microcentrifuge tubes.
- Micro-pipette (10–1000 µL).
- Humid chamber.
- Incubator set at 37°C ± 1°C.
- Fluorescence microscope.
- Demineralized cyst/oocyst-free water.
- Hydrochloric acid (calibrated or standardized stocks must be used).
- Sodium hydroxide solution (calibrated or standardized stocks must be used).
- Methanol.
- Fluorescein isothiocyanate (FITC) conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibody.
- Phosphate buffered saline (PBS).
- 4'6 diamidino-2-phenyl indole (DAPI).
- DABCO/glycerol mounting medium.
- Cover slips.

Note: All reagents should be of analytical grade.

General Guidelines

- Resuspend the Dynabeads® before use to obtain a homogeneous suspension of beads in suspension.
- Shake SL™-Buffer A and B before use. Precautions should be taken to prevent bacterial contamination of opened vials.

Protocols

Prepare Sample

1. Prepare the sample by standard filtration and centrifugation methods to 10 mL.
2. Allow the sample to equilibrate to room temperature.
3. Resuspend the sample in water if it has been suspended in eluting, detergent, or preserving solutions.

Prepare Buffers

1. Use 1 mL of 1X SL™-Buffer A, 1 mL of 10X SL™-Buffer A and 1 mL of 10X SL™-Buffer B for each 10-mL sample, sub-sample, or control.
2. Prepare a 1X dilution of SL™-Buffer A from the 10X SL™-Buffer A (use 100 µL of 10X SL™-Buffer A and dilute to 1 mL with oocyst-free demineralized water).
3. To the same tube containing the 10X SL™-Buffer A, add 1 mL of the 10X SL™-Buffer B.
Note: A crystalline precipitate may form in 10X SL™- Buffer A after prolonged storage at 4°C. Dissolve by equilibrating to room temperature (15°C to 25°C) before use.

Manual Separation of *Cryptosporidium* and *Giardia* Oocysts

The following protocol applies to all water sample concentrates, provided they meet the criteria described previously in "Sample Matrix". To ensure Quality Control, prepare appropriate negative and positive controls to process with test samples.

1. To a flat-sided L-10 tube, add 1 mL of 10X SL™-Buffer A, and 1 mL of 10X SL-Buffer B.
2. Immediately transfer the water sample concentrate to the L-10 tube containing the SL-Buffer. Label the tube with a sample identifier code.
3. Vortex the Dynabeads® anti-Cryptosporidium vial for 10 sec.
4. Thoroughly resuspend the beads by inverting the vial. Add 100 µL of Dynabeads® anti-Cryptosporidium to the L-10 tube containing the water sample concentrate and SL™-Buffer.
5. Vortex the Dynabeads® anti-Giardia vial for 10 sec.
6. Thoroughly resuspend the beads by inverting the vial. Add 100 µL of Dynabeads® anti-Giardia to the L-10 tube.
7. Affix the L-10 tube to a rotating mixer (e.g. MX1) and rotate at 15–20 rpm for 1 hour at room temperature.
8. After rotating for at least 1 hour, remove tube from mixer and place in the MPC™-1 or MPC™-6 with the flat side of the tube facing towards the magnet.
9. Place the magnet side downwards (tube is horizontal and above the magnet).
10. Gently rock the tube end to end approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Continue the tilting action for 2 min.
Note: If the sample is motionless for ≥10 sec, remove the tube from the magnet, and resuspend the beads by gentle shaking, and repeat step 10.
11. Return the magnet to the upright position. Immediately remove the cap and pour off all the supernatant.
12. Remove the tube from the magnet and resuspend sample in 1 mL 1X SL-Buffer A . Mix gently to resuspend all material in the tube.
13. Transfer all the liquid from the L-10 tube to a labelled 1.5-mL microcentrifuge tube.
14. Place the microcentrifuge tube into the MPC™-S, with magnetic strip in place in the vertical position.
15. Without removing the microcentrifuge tube from MPC™-S, gently tilt the tube back and forth 90°. Continue for 1 min with approximately one 90°-tilt per sec.
16. Immediately aspirate the supernatant from the tube and cap held in the MPC™-S. If more than one sample is being processed, conduct three 90° tilt actions before removing the supernatant from each tube.

Dissociate the Dynabeads®-Cysts/-Oocysts Complex

1. Remove magnetic strip from the MPC™-S.
2. Add 50 µL of 0.1 N hydrochloric acid (HCl) to the microcentrifuge tube and vortex for 10 sec.
3. Place the tube in MPC™-S without magnetic strip in place and allow to stand in a vertical position for at least 10 min at room temperature.
4. Vortex for 10 sec.
5. Ensure that all the sample is at the base of the tube. Place microcentrifuge tube in MPC™-S.
6. Insert the magnetic strip in the MPC™-S in the tilted position and allow the tube to stand undisturbed for 10 sec before proceeding to the "Post IMS/AIMS" section.

Automated Immunomagnetic Separation (AIMS)

- For labs using the IDEXX Filta-Max filtration system protocols, begin with a 50-mL concentration, which is then centrifuged according to standard procedure. After centrifugation, the supernatant is removed via gentle aspiration to 3 mL volume. The pellet size is recorded.
- For labs using the Pall Envirochek or Envirochek HV filter capsule, filter and elute the sample as usual. After centrifugation, re-suspend the pellet in 30 mL of buffer and transfer to a 50-mL conical tube. Perform two additional rinses (10 mL each) of the centrifuge tube to recover all remaining sediment. Centrifuge the sample again at 1500 × g. Allow the rotator to coast to a stop; do not use the brake. Record the pellet volume on the bench sheet. After centrifugation the supernatant is removed via gentle aspiration to 3 mL volume. The pellet size is recorded.
Note: If the pellet is above 0.25 mL, then split the sample into two equal sub-samples.

1. Vortex to resuspend the sample.
2. Place one disposable sample tube strip into a BeadRetriever™ rack for each sample to be processed. The tab on the tube strip may be used for sample labeling.
3. To tubes 1–3 add 100 µL of 10X SL Buffer A and 100 µL of 10X SL Buffer B.
4. To tube 4 add 1 mL of 1X SL Buffer A.
5. To tube 5 add 100 µL of 0.1 N HCl.
6. Split the 3-mL sample concentrate across tubes 1–3; 1 mL into each tube 1–3.
7. Add up to 300 µL RO water to the sample concentrate tube. Vortex the concentrate tube to resuspend and wash any debris left behind. This produces the rinsate.
8. Split the rinsate across tubes 1–3; up to 100 µL to each tube 1–3.
9. Add 33 µL of Dynabeads® anti-Cryptosporidium to each tube 1–3. For *Cryptosporidium* only detection, proceed to step 11.
10. For dual *Giardia* and *Cryptosporidium* detection within the same assay, also add 33 µL of Dynabeads® anti-*Giardia* to each tube 1–3.
11. Insert the protective tip combs into the BeadRetriever™.
12. Insert the rack containing the tube strips into the BeadRetriever™.
13. Check that everything is properly aligned and close the instrument door.
14. Select the GC COMBO program by scrolling with the arrow keys and then press the **START** button.
15. During instrument operation the door should be kept closed at all times.
16. The display will show the time left for each stage of the automated IMS protocol. The stages of the GC Combo protocol in order are: mixing stage, bead isolation, wash stage, and then dissociation stage.
17. After the dissociation stage is finished, the machine will beep until the user presses the **STOP** button.
18. At the end of the program run, open the instrument door and carefully remove the rack containing the sample tube strips.
19. Remove each sample tube strip one at a time.
20. Proceed to "Post IMS/AIMS Process".

Post IMS / AIMS Process

Notes:

- Microscope slides may be treated to enhance the binding of organisms. However, some treatments may inhibit the binding of cysts/oocysts concentrated by IMS. To ensure compatibility between slide and IMS separated cysts/oocysts, the use of Spot-On slides is recommended.
- Use of DABCO/glycerol as an anti-fade agent should be treated with caution; some reports indicate that it may be associated with leaching of FITC following staining.
- An indirect monoclonal antibody is not recommended for slide use. Monoclonal antibodies in which a high concentration (>1:10,000) of Evan's Blue is used as a counter-stain are also not recommended.

1. Prepare a Spot-On slide for sample screening. Label the slide appropriately and add 5 µL of 1 N NaOH solution to the sample well.
2. Transfer all fluid from microcentrifuge tube (manual protocol) or sample tube strip (automated protocol) onto the same well of the slide which already contains 5 µL of 1N NaOH. Air-dry the sample onto the slide.
3. Add one drop (50 µL) methanol to each well of the slide and allow to evaporate to dryness at room temperature.
4. Apply 50 µL of a combined fluorescein isothiocyanate (FITC) conjugated anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibody at working dilution to each well of the slide. Ensure complete coverage of each well.
5. Put the slide in a humid chamber and place in an incubator at 37°C for 30 min.
6. Use a Pasteur pipette and gently aspirate the monoclonal antibody from the wells.
7. Apply one drop (50 µL) 4'6-diamidino-2-phenyl indole (DAPI) in PBS solution (0.4 µg DAPI/mL) to each well and allow to stand for 1 min.
8. Use a Pasteur pipette and gently aspirate the DAPI solution from each well.
9. Apply one drop (50 µL) of water to each well and leave for 1–3 sec to remove residual PBS and DAPI solution.
10. Use a Pasteur pipette and gently aspirate the water from each well.
11. Immediately before screening each slide by fluorescence microscopy apply 10 µL DABCO/glycerol mounting medium to each well of the slide, allowing the drop to fall freely (i.e. avoid contact between slide and pipette tip), and apply the cover-slip to the slide. Do not press the cover-slip.

Specificity And Sensitivity

All *Giardia* cysts and *Cryptosporidium* oocysts which react with the antibodies bound to the beads will be isolated using Dynabeads® GC-Combo. This includes cysts of *Giardia* duodenalis (syn lamblia; syn intestinalis) and oocysts of *Cryptosporidium parvum* and *C. hominis*, the species of greatest public health significance, but may also include cysts and oocysts of other species reactive with the monoclonal antibodies (e.g. *G. muris*, *C. baileyi*, *C. muris*). Recognition of the species of *Cryptosporidium* oocyst and *Giardia* cyst separated from the sample concentrate by IMS will be dependant upon recognition of morphometry and morphology of the oocysts and cysts by the user during subsequent screening processes.

Although some degree of non-specific binding may occur with Dynabeads® GC-Combo, this is limited by the use of the SL™-Buffer and, if the procedure described is adhered to, will not affect the ability of the beads to bind to the *Cryptosporidium* oocysts or *Giardia* cysts. The accuracy of the method is determined by seeding experiments. Percentage recovery efficiency of cysts and oocysts might be affected by factors of the water sample concentrate seeded, such as relative turbidity. Recovery efficiencies of between 60 to >95% should be expected from water sample concentrates conforming to the criteria outlined. It is recommended that controls seeded with known cysts and oocyst numbers are processed alongside test samples to give an indication of efficiency.

Factors Affecting Product Performance

In processing all samples, but particularly in turbid samples, the user must practice care not to aspirate and discard the isolated Dynabeads®-organism complexes. Failure to recover the Dynabeads®-organism complexes could result in positive samples not being detected. During Dynabeads®-organism complex magnetic capture it is essential that gentle rocking of the magnet is continued as described. This prevents binding of low mass debris, which is magnetic or magnetizable. The use of flat sided Leighton tubes other than the L-10 tube can result in poor mixing and localisation of Dynabeads®-organism complexes outside the magnetic capture area. This can adversely affect recovery of target organism.

Description of Materials

Dynabeads® anti-Cryptosporidium and Dynabeads® anti-Giardia are uniform, monodisperse, superparamagnetic, 2.8 µm beads with purified antibodies against *Cryptosporidium* oocysts and *Giardia* cysts covalently bound to the surface. The isolation is conducted in SL™-Buffer. This is a two-component buffer system which has been specifically designed for efficient separation of *Cryptosporidium* oocysts from a wide range of water types. The 10X SL™-Buffer A is a clear, colorless solution that needs to be equilibrated to room temperature (15°C to 25°C) before use, and can be stored at room temperature for up to 7 days. The 10X SL™-Buffer B is a magenta solution.

Related Products

Product	Cat. no.
MPC™-1	12001D
MPC™-6	12002D
MPC™-S	A13346
MX1	15907
BeadRetriever™	15950
Spot-On	74004
L-10 Tubes	74003

REF on labels is the symbol for catalog number.

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