applied biosystems
by life technologies

Dynabeads® anti-Cryptosporidium

Catalog nos. 73001, 73011 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 012)

Kit Contents

Kit contents	73001	73011
Dynabeads® anti-Cryptosporidium	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 73001: 10 tests
Capacity for 73011: 50 tests

Dynabeads® anti-Cryptosporidium contains 1.2×10^8 beads/mL in phosphate buffered saline (PBS), pH 7.4 with 0.1% bovine serum albumin (BSA), and 0.02% sodium azide. For more information on 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® anti-Cryptosporidium is intended for selective separation of *Cryptosporidium* oocysts from water sample concentrates using immuno-magnetic separation (IMS), and 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.
Dynabeads® anti-Cryptosporidium are incubated with the water sample concentrate along with SL™-Buffer. The antibodies coated on the beads will selectively bind oocysts within the water sample concentrate and form a complex. The Dynabeads®-organism complexes are separated using a magnetic particle concentrator (MPC™) and subsequently the oocysts are dissociated from the beads.

Intended User

Any scientist who is skilled, equipped, and/or certified for *Cryptosporidium* testing on water and environmental samples may use Dynabeads® anti-Cryptosporidium. The user must be skilled in using conventional microbiological techniques and interpreting results. Screening of slides for enumeration of separated oocysts must be performed by a user who is proficient in recognition of *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 1622 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).
- Micropipette (10–1000 µL).
- Test tubes, glass-ware, loops, swabs, pipettes, microcentrifuge tubes.
- Filters, centrifuges, and other equipment for preparation of the water sample concentrate from the original water sample.
- Vortex mixer.
- 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 standardised 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.

Protocol

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. Retain the 1X dilution of SL™-Buffer A in a labeled vial for use later in the procedure.
4. To a flat-sided L-10 tube, add 1 mL of 10X SL™-Buffer A, and 1 mL of 10X SL™-Buffer B.
   Note: A crystalline precipitate may form in 10 × SL™- Buffer A after prolonged storage at 4°C. Dissolve by equilibrating to room temperature (15°C to 25°C) before use.

Separate *Cryptosporidium* 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. Immediately transfer the water sample concentrate to the L-10 tube containing the SL™-Buffer. Label the tube with a sample identifier code.
2. Vortex the Dynabeads® anti-Cryptosporidium vial for 10 sec.
3. Resuspend the beads completely by inverting the vial. Add 100 µL of Dynabeads® anti-Cryptosporidium to the L-10 tube.
4. Affix the L-10 tube to a rotating mixer (e.g. MX1) and rotate at 15–20 rpm for 1 hour at room temperature.
5. Place in the MPC™-1 or MPC™-6 with the flat side of tube facing towards the magnet.
6. Without removing the tube from the magnet, place the magnet side of the MPC™-1 downwards (tube is horizontal and above the magnet).
7. Gently rock the tube end to end through approximately 90°, tilting the cap-end and base-end of the tube up and down in turn. Tilt for 2 min with approximately one tilt per sec.
   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 7.
8. Return the magnet to the upright position, tube vertical, with the cap at the top. Remove the cap and pour off all the supernatant.
9. Remove the tube from the magnet and resuspend the sample in 1 mL 1X SL™-Buffer A. Mix gently to resuspend all material in the tube.
10. Transfer all the liquid and beads from the L-10 tube to a labeled 1.5-mL microcentrifuge tube.
11. Place the microcentrifuge tube into the MPC™-S, with magnetic strip in place in the vertical position.
12. Without removing the microcentrifuge tube from the MPC™-S, gently tilt the MPC™-S back and forth 90°. Continue for 1 min with approximately one 90°-tilt per sec.
13. 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° back-and-forth motions 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 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 the 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 about 10 sec.
7. 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.
8. Transfer all fluid from microcentrifuge tube onto the same well of the slide that already contains 5 µL of 1 N NaOH. Be careful not to disturb beads at back-wall of tube. Ensure that all the fluid is transferred.
9. Air-dry the sample onto the slide for staining and proceed to "Staining".

Staining

Notes:

- Microscope slides may be treated to enhance the binding of organisms. However, some treatments may inhibit the binding of oocysts concentrated by IMS. To ensure compatibility between slide and IMS separated 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. Add one drop (50 µL) methanol to each well of the slide and allow it to evaporate to dryness at room temperature.
2. Apply 50 µL of a combined FITC conjugated anti-*Cryptosporidium* monoclonal antibody at working dilution to each well of the slide. Ensure complete coverage of each well.
3. Put the slide in a humid chamber and place in an incubator at 37°C for 30 min.
4. Use a Pasteur pipette and gently aspirate the monoclonal antibody from the wells.
5. 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.
6. Use a Pasteur pipette and gently aspirate the DAPI solution from each well.
7. Apply one drop (50 µL) of water to each well and leave for 1–3 sec to remove residual PBS and DAPI solution.
8. Use a Pasteur pipette and gently aspirate the water from each well.
9. 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 cover-slip to slide. Do not press the cover-slip.

Specificity And Sensitivity

All *Cryptosporidium* oocysts which react with the antibodies bound to the beads will be isolated using Dynabeads® anti-Cryptosporidium. This includes oocysts of *Cryptosporidium parvum* and *C. hominis*, the species of greatest public health significance, but may also include oocysts of other species reactive with the monoclonal antibodies (e.g. *C. baileyi*, *C. muris*). Recognition of the species of *Cryptosporidium* oocyst separated from the sample concentrate by IMS will be dependant upon recognition of morphometry and morphology of the oocysts by the user during subsequent screening processes. Although some degree of non-specific binding may occur with Dynabeads® anti-Cryptosporidium, 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. The accuracy of the method is determined by seeding experiments. Percentage-recovery-efficiency of 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 oocyst numbers are processed alongside test samples to give an indication of efficiency.

Factors Affecting Product Performance

Provided that the amount of particulate matter in the water sample concentrate does not exceed the limits given above, Dynabeads® anti-Cryptosporidium will perform satisfactorily in any water sample concentrate of water destined for potable supply, whether treated or untreated. 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 capture it is essential with continuous tilting and mixing of the magnet. 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 localization of Dynabeads®-organism complexes outside the magnetic capture area. This can adversely affect recovery of target organism. Do not vortex after the capture processes have been completed. Vortex only for dissociation. The use of vacuum aspirators has been shown to significantly reduce the recovery of oocysts. The entire IMS procedure should be performed at room temperature (15°C to 25°C).

Description of Materials

Dynabeads® anti-Cryptosporidium are uniform, mono-disperse, superparamagnetic, 2.8 µm beads with purified antibodies against Cryptosporidium oocysts covalently bound to the surface. 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
Sample Mixer	94701
Spot-On	74004
L-10 tube	74003

REF on labels is the symbol for catalog number.

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