applied biosystems
by life technologies

Dynabeads® anti-Listeria

Catalog no. 71006 **Store at 2˚C to 8˚C**

Rev. Date: August 2012 (Rev. 005)

Product Contents

Product contents	Volume
Dynabeads® anti-Listeria	5 mL

Dynabeads® anti-Listeria is supplied in phosphate buffered saline (PBS) pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.
Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product Description

Intended Use

Dynabeads® anti-Listeria is designed for a rapid isolation and concentration of *Listeria* directly from pre-enriched samples using immunomagnetic separation (IMS). An aliquot of the pre-enriched sample is incubated with Dynabeads® anti-Listeria and the antibodies coated onto Dynabeads® will specifically bind *Listeria* and form a complex. The Dynabeads®-Listeria complexes are subsequently separated and isolated from the sample matrix using a magnetic particle concentrator, MPC™-S.

Intended User

Any user who is skilled in using conventional microbiological techniques, equipped, and/or certified to do *Listeria* testing on food, feed, and environmental samples may use Dynabeads® anti-Listeria. The user must be skilled in using conventional microbiological techniques and interpreting results.

Sample Matrix

IMS with Dynabeads® anti-Listeria can be done on any food, feed, or environmental sample that has been pre-enriched for 24 hours in Half Fraser broth. Environmental samples include waterfiltrate, surface swab streaks, or faecal swabs of animal origin. Food is defined as material used for human consumption, and feed is defined as material used for animal consumption.

Interpretation Criteria

The test is based on plating the concentrated Dynabeads®-Listeria complexes onto internationally accepted *Listeria* selective media, for example Palcam and modified Oxford agars. Chromogenic *Listeria* plating media may also be used to supplement colony identification. Interpretation of presumptive results depends on the skill of the user to correctly differentiate the isolated colonies based on the typical *Listeria* morphology. Suspect colonies must be confirmed using standard biochemical and serological test methods.

Required Materials

- Magnets: MPC™-6, MPC™-1, MPC™-S.
- Mixer allowing tilting and rotation of tubes (e.g. MX 1, Sample Mixer).
- Micropipette (10–100 µL).
- 1-mL dispenser pipette.
- Half Fraser broth; commercially available from all major media manufacturers.
- Stomacher and stomacher bag with filter.
- Test tubes, glass-ware, loops, swabs, and pipettes.
- Wash buffer (PBS-Tween®): 0.15 M NaCl, 0.01 M Sodium Phosphate buffer, pH 7.4 with 0.05% Tween®-20. (Autoclave at 121°C for 15 min, store at 2°C to 8°C.)

General Guidelines

- Perform the "Manual IMS procedure" on a benchtop at room temperature ranging from 18°C to 28°C.
- Automated IMS could be performed using the BeadRetriever™, in which case all performance parameters have been fully optimized and therefore are not dependent on operator aptitude.

Protocol

The following protocol applies to all samples. All of the discarded material should be placed in appropriate microbiological containers and autoclaved.

Prepare Sample

Food Samples

1. Weigh 25 grams of sample material and place into a stomacher bag with filter and add 225 mL of Half Fraser broth. A stomacher bag with filter removes particulate material and fatty substances, which are inhibitory to IMS. (For certain foods, e.g., meat with bones or dry pasta, a blender is preferred prior to using a stomacher bag with a filter to avoid the risk of perforation.)
2. Incubate the prepared sample in the stomacher bag for 24 hours at 30°C.
3. Mix the stomacher bag pre-enriched samples thoroughly by homogenizing once more. Pipet 1 mL aliquot from the filtered section for the IMS procedure according to the "Manual IMS" section.

Environmental Samples

1. Take a swab sample from any surface material or filter 10 L of water through a membrane filter.
2. Place the swab or filter into an appropriate container filled with 10–50 mL of pre-enrichment broth. Incubate for 24 hours at 30°C.
3. Mix by shaking vigorously and pipette 1 mL aliquot for the IMS procedure according to the "Manual IMS" section .

Manual IMS

1. Remove the magnetic plate and load the necessary number of 1.5-mL microcentrifuge tubes into the MPC™-S.
2. Resuspend Dynabeads® anti-Listeria until the pellet in the bottom of the vial disappears by using a vortex machine. Pipet and dispense 20 µL into each microcentrifuge tube.
3. Add the 1 mL from the pre-enriched sample aliquot according to the "Sample Preparation" section, step 3 and close the tube. Change to a new pipette for each new sample.
4. Invert the MPC™-S rack five times. Incubate at room temperature for 10 min with gentle continuous agitation to prevent the beads from settling (e.g. in a MX sample mixer).
5. Insert the magnetic plate into the MPC™-S. Invert the rack several times to concentrate the beads into a pellet on the side of the tube. Allow 3 min for proper recovery of beads.
6. Open the tube cap using the tube opener provided and carefully aspirate and discard the supernatant, as well as the remaining liquid in the tube cap. Change to a new pipette for each new sample.
7. Remove the magnetic plate from the MPC™-S.
8. Add 1 mL of wash buffer. Do not touch the tube with the pipette since this can cross-contaminate the samples and the wash buffer. Close the tube cap. Incubate at room temperature with gentle continuous agitation for another 10 min.
9. Repeat steps 5–7 once.
10. Resuspend the Dynabeads®-Listeria complexes in 100 µL of wash buffer and mix vigorously using a vortex mixer.

Plating

The resuspended Dynabeads®-Listeria complexes are now ready for plating. Transfer 50 µL onto two *Listeria* plating media and plate by standard streaking with a loop or the swab-streak technique. All inoculated plating media must be incubated at 37°C. The plates are read after 24 hours and if necessary after 48 hours for presumptive *Listeria* colonies. Total analysis time from sample receipt to presumptive results is 48 hours.

Confirmation

The presumptive *Listeria* colonies must be confirmed by standard biochemical and serological testing or by genetic fingerprinting to identify the species. Dynabeads® anti-Listeria may record false negative results if bead recovery was particularly low and/or the level of *Listeria* species present were below 1000 cells/mL of enriched sample. Following good laboratory practices, false positive results do not occur since the possibility to verify presumptive colonies is always applicable.

Specificity And Sensitivity

The recommended protocols for use with Dynabeads® anti-Listeria will determine the presence or absence of one viable *Listeria* in 25 g of sample if this one cell is able to replicate and is not obstructed by resident background flora during the 24 hours enrichment. Dynabeads® anti-Listeria enables visible growth of *Listeria* on a plating medium from an enriched sample containing as low as 100 *Listeria*/mL against a background of competing flora greater or equal to 106 organisms/mL. Dynabeads® anti-Listeria significantly concentrates *Listeria* from a mixed culture. For example, an initial ratio of *Listeria* versus competing flora of 1:20 is often reduced to between 1:1 to 1:2 giving a positive concentration factor ranging between 10 to 20 times. A certain degree of cross-reactivity and non-specific binding is evident, but it does not affect the overall ability of the product to bind *Listeria* in a mixed culture. The accuracy of the method is not measurable since IMS is a qualitative method. More than one *Listeria* may be bound to one or more beads and form aggregates. These Dynabeads®-Listeria aggregates may give rise to only one colony-forming unit on the selective plating media. It is therefore important to vortex vigorously to break up aggregates prior to plating. Precision of the method is dependent on the extent to which particles are recovered from different sample matrices.

Factors Affecting Product Performance

- The performance of Dynabeads® anti-Listeria is dependent on the extent of particle recovery from different sample matrices. Failure to recover the Dynabeads®-Listeria complexes results in failure to detect the presence of *Listeria* in an otherwise positive sample.
- Do not aspirate and discard the isolated Dynabeads®-Listeria complexes. To prevent loss of these complexes, leave approximately 100 µL of the original sample in the tube and dilute further by adding 1 mL of wash buffer (step 6–8 of the "Manual IMS" protocol). Follow the remaining processing steps as described.
- In extremely fatty, viscous, and/or particulate samples a two-fold dilution of the 24-hour enriched sample with the wash buffer must be made prior to IMS analysis. Such a dilution will not limit detection of *Listeria* but rather ensure that Dynabeads® are recovered.
- Perform the Manual IMS procedure on a benchtop at room temperature ranging from 18°C to 28°C.
- Alternatively, perform automated IMS using the BeadRetriever™, in which case all performance parameters have been fully optimized and therefore are not dependent on operator aptitude.

Description of Materials

Dynabeads® anti-Listeria is made of uniform, paramagnetic, polystyrene beads and purified anti *Listeria* antibodies, which are bound covalently onto the surface. Dynabeads® anti-Listeria reacts against all *Listeria monocytogenes* serotypes but shows a reduced reaction to all other *Listeria* species.

Related Products

Product	Cat. no.
MPC™-1	12001D
MPC™-6	12002D
MPC™-S	A13346
MX1	15907
Sample Mixer	94701
BeadRetriever™	15950
BeadRetriever™ Tubes and Tips	15951

REF on labels is the symbol for catalog number.

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