invitrogen™

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Dynabeads® anti-Listeria

For rapid selective isolation and concentration of Listeria

For laboratory use only.

Not for use in human diagnostic procedures.

PRODUCT DESCRIPTION

Dynabeads® anti-Listeria is made of uniform, paramagnetic, polystyrene beads and purified anti-Listeria antibodies, which are bound covalently onto the surface. The antibody coated beads are supplied in a suspension of phosphate buffered saline (PBS) pH 7.4 with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN_3).

PRINCIPLE

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, Dynal® MPC™-S.

INTENDED USER

Any laboratory 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 water filtrate, 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.

ANALYTE

Dynabeads anti-Listeria reacts against all *Listeria* monocytogenes serotypes but shows a reduced reaction to all other *Listeria* species.

INTERPRETATION CRITERIA

The test is based on either 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.

Factors that affect the products 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 could result in failure to detect the presence of *Listeria* in an otherwise positive sample. In extremely fatty, viscous and/or particulate samples a two-fold dilution of the 24 hours 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. The user must practice care not to 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, protocol B). Follow the remaining processing steps as described. The entire IMS procedure (see protocol B) shall be performed on a bench top at room temperature ranging from 18-28°C. Alternatively, automated IMS could be performed using the BeadRetriever™, in which case all performance parameters have been fully optimised and therefore are not dependent on operator aptitude.

INSTRUCTIONS FOR USE

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

A. Sample preparation

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, for example meat with bones or dry pasta, a blender is preferred prior to using a stomacher bag to avoid the risk of perforation. After blending, the contents should be transferred into a stomacher bag with a filter).
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 homogenising once more. Pipette 1 ml aliquot from the filtered section for the IMS procedure in Section B.

Environmental samples

1. Take a swab sample from any surface material or filter 10 litres 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 in section B.

B. Immunomagnetic separation (IMS)

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

C. Isolation procedure

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.

D. Confirmation

The presumptive *Listeria* colonies must be confirmed by standard biochemical and serological testing or by genetic fingerprinting to identify the species.

False negative/positive results

Dynabeads anti-Listeria may record false negative results if bead recovery was particularly low and/or the level of *Listeria* species present were below 1,000 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 grams of sample if this one cell is able to replicate and nor competed out 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.

Accuracy and precision

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.

MATERIALS NOT PROVIDED

- Micro-pipette (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 the buffer at 121°C for 15 minutes). Prepared buffer can be stored under refrigeration
- Selective culture media

All reagents should be of analytical grade.

GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001: 2000 and ISO 13485:2003.

STORAGE/STABILITY

Dynabeads anti-Listeria is stable, when stored unopened at 2-8°C, until the expiration date stated on the label.

PRECAUTIONS/LIMITATIONS

In order to obtain a homogeneous dispersion of beads in suspension, resuspend Dynabeads anti-Listeria by using a vortex until pellet in the bottom disappears before use.

Precautions should be taken to prevent bacterial contamination of opened vials. All material that is used and contaminated should be autoclaved and properly disposed of according to local regulations.

Avoid pipetting by mouth. This product contains 0.02% sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build-up.

The product is not for use in human diagnostic or therapeutic procedures.

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