invitrogen™

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Dynabeads® anti-Legionella

For rapid, selective enrichment of *Legionella* spp.

For research use only

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1 PRODUCT DESCRIPTION

1.1 Intended Use

For rapid selective separation of *Legionella* species (*spp.*) from water samples and water concentrates. This process can be automated using a Invitrogen's BeadRetriever™ bench top instrument or performed using a manual method.

1.2 Intended User

Any laboratory skilled in using conventional microbiological techniques, equipped and/or certified to do *Legionella* testing on water and environmental samples may use Dynabeads anti-Legionella. The user must be skilled in using conventional microbiological techniques and interpreting results.

1.3 Sample Matrix

Water samples and water sample concentrates.

1.4 Principle

Dynabeads anti-Legionella is designed for the rapid concentration of *Legionella* directly from samples using immunomagnetic separation (IMS). This product, depending on the type of sample analysed, supplements the use of traditional selective media for the isolation of *Legionella* spp. from environmental, potable and domestic water samples. Dynabeads anti-Legionella is simply incubated with an aliquot of the sample or sample concentrate and the antibodies coated onto the beads will specifically bind the target bacteria. The bead-bacteria complexes are subsequently separated by applying a magnetic field. The whole IMS process can be automated using a BeadRetriever instrument or performed manually.

1.5 Interpretation Criteria

The test is based on plating the concentrated bead-bacteria complexes onto *Legionella* selective media (for example GVPC agar) using a standard plating technique. Interpretation of presumptive results depends on the skill of the user to correctly differentiate the isolated colonies based on the typical *Legionella* morphology. Suspect colonies must be confirmed by standard biochemical and serological test methods.

1.6 Description of Materials

Dynabeads anti-Legionella are uniform, superparamagnetic, polystyrene microscopic beads with antibodies against Legionella covalently bound to the surface. The 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).

Sufficient Dynabeads anti-Legionella are provided to perform 100 tests.

Additional Materials & Equipment Required & Supplied By Invitrogen

For performing AIMS:

Component	Cat. no.	Pack Size
BeadRetriever instrument A bench top instrument for performing automated IMS	159.50	1 Unit
BeadRetriever Tubes and Tips Disposable tubes and magnetic tips for use in the BeadRetriever	159.51	240 Tests

For performing manual IMS:

Component	Cat. no.	Pack Size
Dynal Magnetic particle concentrator™(MPC)-S	120.20D	1 Unit
Dynal-MX4	159.10	1 Unit
Dynal Sample Mixer (US only)	947.01	1 Unit

Additional Materials & Equipment Needed Not Supplied By Invitrogen

- Micro pipette (10-100 µl)
- 1 ml dispenser pipette
- Disposable loops, swabs and pipettes
- Selective culture media such as GVPC, MWY and BMP
- Sample Buffer: **10x PBS-Tween** is composed of 1.5M NaCl, 0.1 M Sodium Phosphate buffer, pH 7.4 with 0.5% Tween 20. Autoclave at 121°C for 15 minutes.
- Wash Buffer: **1x PBS-Tween** is composed of 0.15M NaCl, 0.01 M Sodium Phosphate buffer, pH 7.4 with 0.05% Tween 20. Autoclave at 121°C for 15 minutes.

NOTE: All reagents should be of analytical grade. Prepared wash buffers can be stored under refrigeration.

2 PROTOCOLS

For quality control, users should prepare appropriate negative and positive samples to process alongside the test samples.

***L. pneumophila* serogroup 1 (NCTC 12821) is a suitable strain for use as a positive control organism. This culture is available for purchase from the National Collection of Type Cultures, HPA, London, UK (http://www.hpa.org.uk/srmd/div_cdmssd_nctc/index.htm)**

2.1 Sample Preparation

This test protocol has been designed for use with 1 ml of water sample concentrates, spiked water samples, environmental swab samples and dilute suspensions of target, non-target or reference organisms. This protocol is also suitable for samples which have been acid or heat treated.

If the sample has been stored below room temperature leave sufficient time to allow the sample to equilibrate to room temperature.

Samples may be prepared by standard filtration and/or centrifugation methods appropriate to meet local regulations.* This is recommended where target cell numbers are expected to be below 1000 colony forming units (cfu) per Litre.

Environmental swab samples may be eluted into a suitable volume of Maximum Recovery Diluent prior to IMS.

NOTE: Maximum Recovery Diluent is an isotonic diluent containing a low level of peptone used for maintaining the viability of organisms during dilution procedures. Please use Oxoid Cat. No. CM733, Difco Cat. No. 218971 Dehydrated – 500 g, or equivalent.

***BS ISO 11731-2:2004, BS 6068-4.18:2004** Water quality. Detection and enumeration of Legionella. Direct membrane filtration method for waters with low bacterial counts

BS 6068-4.12:1998, ISO 11731:1998 Water quality. Microbiological methods. Detection and enumeration of *Legionella* (Current, Work In Hand) http://bsonline.techindex.co.uk

2.2 Performing The Immunomagnetic Separation

2.2.1 Automated Immunomagnetic Separation (AIMS) Using Dynabeads Anti-Legionella & BeadRetriever

NOTE: Please carefully read the instrument operating instructions of the BeadRetriever prior to its use.

Place one disposable sample tube strip into a BeadRetriever rack for each sample to be processed and using aseptic technique, dispense reagents into each tube. The tab on the tube strip may be used for labelling samples.

1. Add 100 µl of Sample buffer to sample tube 1.
2. Vortex Dynabeads anti-Legionella for a minimum of 10 seconds to mix.
3. Add 50 µl of Dynabeads anti-Legionella into sample tube 1.
4. Add 1 ml of Wash buffer to tubes 2 and 3 within the strip.
5. Add 100 µl of Wash buffer to tube 4.
6. Add 1 ml of sample aliquot to tube 1 (to avoid cross contamination between samples it is recommended that sample transfer is performed at least 1 metre from the other prepared tube strips).
7. Insert the sterile disposable protective tip combs into the instrument. Each comb should click into position.
8. Insert the rack with filled tubes into the correct position in the instrument.
9. Check that everything is properly aligned and close the instrument door.
10. Select the Environmental program sequence by scrolling with the arrow key and press the START button.
11. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
12. At the end of the program run, remove the tube rack from the instrument and plate the bead-bacteria complexes from tube 4 onto an appropriate plating media according to local procedures.
13. Culture plates should be incubated according to standard procedures.
14. Remove the tip combs and tube strips and discard into an appropriate biohazardous waste container and autoclave prior to disposal.

Safety Note: During operation the BeadRetriever does not create a biohazardous aerosol and may be operated safely on the laboratory bench. For further information see: http://www.hpa.org.uk/srmd/div_esl_su/pdf_guidance2.htm.

2.2.2 Immunomagnetic Separation - Manual Method

1. Remove the magnetic plate and load the necessary number of 1.5 ml Eppendorf tubes into the Dynal MPC-S.
2. Add 100µl of Sample buffer into each test tube.
3. Vortex Dynabeads anti-Legionella for a minimum of 10 seconds to mix.
4. Add 50 µl of Dynabeads anti-Legionella into each sample tube.
5. Add a 1 ml sample aliquot and close the tube. To avoid cross contamination between samples it is recommended that only one sample tube is opened at any one time.
6. Change to a new pipette for each new sample.
7. Invert the Dynal MPC-S rack five times. Incubate at room temperature for 35 minutes with gentle continuous agitation to prevent the beads from settling (e.g. in a Dynal MX4 mixer or a Dynal Sample Mixer (US only).
8. Insert the magnetic plate into the Dynal MPC-S. Note: The magnetic strip should be inserted into the vertical position (Refer to the Instructions for Use for the Dynal MPC®-S). Allow 3 minutes for complete recovery of beads. During this period, invert the rack several times in order to concentrate the beads into a pellet on the side of the tube.
9. Open the tube cap and carefully aspirate and discard the supernatant as well as the remaining liquid in the tube's cap. (Refer to Section 2.4 Factors that affect the performance of the product). Change to a new pipette for each new sample.
10. Remove the magnetic plate from the Dynal MPC-S.
11. Add 1 ml of Wash buffer. 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. Invert the rack several times to resuspend the beads.
12. Repeat steps 8-11.
13. Repeat steps 8-10.
14. Resuspend the bead-bacteria complex in 100 µl of Wash buffer and plate onto an appropriate plating media according to local procedures.
15. Culture plates should be incubated according to standard procedures.
16. Discard used tubes and tips into an appropriate biohazardous waste container and autoclave prior to disposal.

2.3 Specificity And Sensitivity

Dynabeads anti-Legionella reacts with *Legionella pneumophila* including serogroup 1 strains and *L .bozemanii, L. brunensis, L. dumoffii, L. micdadei* and *L. anisa*.

The Dynabeads anti-Legionella protocol is able to recover viable *L. pneumophila* cells from inocula containing less then 100 colony forming units per millilitre of test sample in clean water.

2.4 Factors Which Affect The Product's Performance

- The IMS procedure should be performed on a bench-top at room temperature between 15-25°C and all reagents must be at room temperature before use.
- Ensure that the Dynabeads anti-Legionella are fully dispersed by vigorous vortexing for at least 10 seconds before use.
- When performing manual IMS the user must practice care not to aspirate and discard the isolated bead-bacteria complexes. The use of vacuum aspirators has been shown to reduce the recovery of bacteria. Failure to recover the bead-bacteria complexes could result in failure to isolate *Legionella* spp.

- During bead-bacteria complex magnetic capture it is essential that gentle rocking of the Dynal MPC's is continued. This prevents binding of low mass debris, which is magnetic or magnetisable.

2.5 Precautions/Limitations

In order to obtain a homogeneous dispersion of beads in suspension, resuspend Dynabeads anti-Legionella 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.

3 GENERAL INFORMATION

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

Storage/Stability

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

Technical Service

Please contact Invitrogen for further technical information at http://www.invitrogen.com/contact.

A certificate of Analysis (CoA) is available on request.

Warnings & Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Material Data Safety Sheet is available from http://www.invitrogen.com.

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The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen's expense, of any products which are defective in manufacture, and which shall be returned to Invitrogen's, transportation prepaid, or at Invitrogen's option, refund of the purchase price.

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4 REFERENCES

BS ISO 11731-2:2004, BS 6068-4.18:2004 Water quality. Detection and enumeration of *Legionella*. Direct membrane filtration method for waters with low bacterial counts.

Invitrogen Dynal is a part of the Invitrogen Group.

Contact details for your local Invitrogen sales office/technical support can be found at http://www.invitrogen.com/contact

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