invitrogen™

Cat. no. 710.02

Rev. no. 008

Dynabeads® anti-Salmonella

For rapid, selective enrichment of Salmonella

For research use only

INDEX

1 PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads anti-Salmonella is designed for rapid, selective concentration of Salmonella directly from pre-enriched samples. This process can be automated using a 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 Salmonella testing on food, feed and environmental samples may use Dynabeads anti-Salmonella.

The user must be skilled in using conventional microbiological techniques and in interpreting results.

1.3 Sample Matrix

Any food, water, feed or environmental sample that has been pre-enriched for 18-24 hours in a standard Salmonella pre-enrichment broth is suitable for IMS with Dynabeads anti-Salmonella.

1.4 Principle

Dynabeads anti-Salmonella is designed for rapid, selective concentration of Salmonella directly from pre-enriched samples using manual IMS or automated IMS on the Dynal BeadRetriever™.

Dynabeads anti-Salmonella may either replace or supplement the use of a selective enrichment broth stage for the isolation of Salmonella. Dynabeads anti-Salmonella are simply incubated with an aliquot of the pre-enriched sample and the antibodies coated onto the beads will specifically bind the target bacteria. The bead-bacteria complexes are subsequently separated by using a magnetic particle concentrator, Dynal MPC®-S. For automated IMS, the Dynabeads anti-Salmonella, wash buffers and samples are loaded into the BeadRetriever and all incubations and wash steps are carried out automatically in the instrument.

After IMS, Dynabeads anti-Salmonella can be used with any standard Salmonella selective plating medium to accommodate the different Salmonella testing regimes used from country to country.

Concentrated bead-bacteria complexes can be processed using either a RAPID method or an ENHANCED method.

The RAPID method is recommended for processed samples containing low resident flora. Presumptive identification is achieved 24 h sooner than with the ENHANCED method. Following the IMS process the bead-bacteria complexes are plated directly onto internationally accepted Salmonella selective media, such as Brilliant Green agar (BGA), Xylose-Lysine-Deoxycholate agar (XLD), Bismuth Sulphite agar (BSA), Hektoen agar (HE), etc.

The ENHANCED method improves the isolation of Salmonella from samples containing high resident flora. The method consists of transferring the bead-bacteria complexes into standard selective enrichment broth and then plating onto any of the above media or other chromogenic Salmonella plating media (e.g. Rambach agar) using the standard plating technique

The improved sensitivity of the ENHANCED method is due to the specific concentration of Salmonella in the pre-enriched sample during IMS and the significant lowering of the initial ratio between Salmonella species (spp.) and background flora. The subsequent transfer of the bead-bacteria complex into Rappaport Vassilliadis Soya peptone broth (RVS) gives the Salmonella spp. a growth advantage due to the further inhibition of the competitive flora.

The ENHANCED method can be used for all food categories, except shell eggs (see section 2.1). Refer to the national reference method for analysing Salmonella (i.e. BAM, ISO etc.) when a particular food material (e.g. cocoa powder, spices etc.) requires special sample treatment and incubation media. The special sample treatment will not interfere with IMS but will only enhance the detection of Salmonella in these particular samples.

For both methods the recommended swab-streak technique should be used when plating the bead-bacteria complexes as this will result in improved colony isolation on culture media.

1.5 Interpretation Criteria

Interpretation of presumptive results depends on the skill of the user to correctly differentiate the isolated colonies based on typical Salmonella morphology. Suspect colonies must be confirmed by standard biochemical and serological test methods.

1.6 Description of Materials

Dynabeads anti-Salmonella are uniform, superparamagnetic, polystyrene microscopic beads with affinity purified antibodies against Salmonella 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-Salmonella are provided to perform 250 tests (Product Number 710.02).

Additional Materials & Equipment Required & Supplied By Invitrogen Dynal

For performing AIMS:

Component	Prod No.	Pack Size
BeadRetriever instrument A bench top instrument for performing automated IMS	159.50	1 Unit
BeadRetriever Tubes and Tips Disposable sample tube strips and tip combs to protect the magnetic probes for use in the BeadRetriever	159.51	240 Tests

For performing manual IMS:

Component	Prod 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

All reagents should be of analytical grade.

Additional Materials & Equipment Needed & Not Supplied By Invitrogen Dynal

- Micropipette (10 - 100 µl)
- 1 ml dispenser Pipette
- Stomacher apparatus and stomacher bag with filter
- Test tubes, glassware, loops, swabs
- Washing buffer (PBS Tween): 0.15M NaCl, 0.01M Sodium-Phosphate buffer, pH 7.4, with 0.05 % Tween-20. (Autoclavable at 121°C for 15 minutes)
- Pre-enrichment broths such as buffered peptone water (BPW)
- Enrichment and selective culture media

All reagents should be of analytical grade.

2 PROTOCOLS

2.1 Sample Preparation

Weigh 25 g of sample material and place into a stomacher-bag with filter and add 225 ml of pre-enrichment broth. (Invitrogen Dynal recommends buffered peptone water as a pre-enrichment broth)

Mix well using the stomacher apparatus (A stomacher-bag with filter removes particulate matter as well as fatty components and allows easy pipetting of clear aliquot for analysis). For certain foods, for example bony meat, pasta, etc. a blender is preferred prior to using a stomacher bag to avoid the risk of perforation. However, after blending the contents should be transferred into a stomacher bag with a filter.

For environmental samples using a swab, place the swab into 10-50 ml of pre-enrichment broth and incubate as described below.

Incubate the prepared sample in the stomacher bag for 18-24 hours at 37°C. Mix the pre-enriched sample thoroughly by homogenising once more. Pipette 1 ml aliquot of the filtered suspension for the immunomagnetic separation procedure. Change to a new pipette for each new sample.

Method For Shell Eggs

Wash dirty eggs with a stiff brush under running water, and dry with a paper towel. Dip the eggs into 70% ethanol for 5-10 seconds and allow to dry. Alternatively follow any standard procedure for disinfecting shell eggs.

Aseptically crack open the eggs and mix/blend thoroughly both white and yolk. Add Ferrous Sulphate ($FeSO_4$) solution to a final concentration of 35 mg/L. Pre-incubate the egg mixture at 37°C for 6 hours.

After pre-incubation, mix the egg mixture thoroughly. Dilute an aliquot five fold with wash buffer or buffered peptone water and use 1 ml of this dilution for IMS analysis. Use a new pipette or a new pipette tip for each sample to avoid cross-contamination. Re-incubate the remaining undiluted egg mixture overnight at 37°C.

2.2 Performing The Immunomagnetic Separation

2.2.1 Automated Immunomagnetic Separation (Aims) Using Dynabeads Anti-Salmonella & BeadRetriever

1. Load one BeadRetriever sample tube strip for each sample into a sample rack.
2. Resuspend Dynabeads anti-Salmonella by vortexing until the pellet in the bottom of the tube disappears and aseptically add 10 µl of properly mixed Dynabeads anti-Salmonella into sample tubes 1 and 2.
3. Aseptically add 500 µl of wash buffer to sample tubes 1 and 2.
4. Aseptically add 1 ml of wash buffer to tubes 3 and 4 within the strip.
5. Aseptically add 100 µl of wash buffer to tube 5.
6. For each sample remove the labelled sample tube strip from the sample rack and place in a second sample rack (one metre away). Add 500 µl of the test sample to tubes 1 and 2 and return the inoculated tube to the first sample rack. Repeat for the remaining samples.
7. Aseptically insert the sterile protective sample tip combs into the instrument.
8. Insert the rack with filled tubes into the instrument to lock it in place.
9. Check that everything is properly aligned and close the instrument door.
10. Select the SALMONELLA program sequence by scrolling with the arrow key and press the START button.

NOTE: For the Shell Eggs method select the Salmonella (eggs) program from the BeadRetriever menu.

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 sample rack from the instrument and, for each sample, process the bead-bacteria complexes either using the RAPID or ENHANCED methods described below.
13. Remove the sample tip combs and discard into a biohazard waste container together with the tube strips.

2.2.2 Immunomagnetic Separation – Manual IMS

NOTE: To avoid cross-contamination and for safety reasons, it is strongly recommended that IMS should be performed using the BeadRetriever. In the absence of the BeadRetriever, strict adherence to good laboratory practice and the following instructions are a prerequisite to obtaining valid results.

1. Remove the magnetic plate and load one 1.5 ml Eppendorf tube for each sample into the Dynal MPC-S.
2. Resuspend Dynabeads anti-Salmonella by vortexing until the pellet in the bottom of the tube disappears by pipette and dispense 20 µl into each tube.
3. Add 1 ml of the pre-enriched filtered sample aliquot and close the tube. Change to a new pipette for each new sample.
4. Invert the Dynal MPC-S rack five times to mix sample and beads. Incubate at room temperature for 10 minutes with gentle continuous agitation to prevent the beads from settling (e.g. in a Dynal MX4 sample mixer).
5. Insert the magnetic plate into the Dynal MPC-S. Allow 3 minutes for proper 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.

NOTE: The magnetic plate for the Dynal MPC-S has two positions for insertion. The vertical position is intended for use with round-bottomed or conical microcentrifuge tubes for larger volume applications (0.5 - 2 ml). The tilted position is for conical microcentrifuge tubes only and is better for lower volume applications (0.01 - 0.5 ml).

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's cap taking care not to disturb the pellet of IMS beads on the side wall of the tube. 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. Change to a new pipette for each new sample. Do not touch the tube with the pipette since this can cross-contaminate the samples as well as the wash buffer. Close the cap. Invert the rack several times to resuspend the beads.
9. Repeat steps 5-8.
10. Repeat steps 5-7.
11. Resuspend the Dynabead-bacteria complex in 100 µl of wash buffer. Mix briefly using a vortex mixer
12. For each sample process the bead-bacteria complexes using either the RAPID or ENHANCED methods described below.

2.2.3 Post IMS

The ENHANCED Method

This is the recommended method for all food and environmental samples. Presumptive Salmonella positive results are available three days after receipt of samples.

Transfer the concentrated, resuspended bead-bacteria complexes into 10 ml of Rappaport Vassilliadis Soya peptone broth (RVS) and incubate at 42°C for 18-24 hours.

Follow standard procedure for isolation by spreading a loopful of RVS culture onto any Salmonella plating media.

The RAPID Method

This is recommended for processed or foods known to harbour none or low levels of background flora only. Presumptive Salmonella positive results are available two days after receipt of samples.

Transfer 50 µl of the resuspended bead-bacteria complex onto each of two Salmonella selective agar plates. (BGA, XLD, BSA, HE etc.)

2.2.4 Dynabeads anti-Salmonella IMS For Shell Eggs

Both the Automated and Manual IMS methods are suitable for analysis of enrichment cultures from samples of raw Shell Egg.

For the automated method, follow the instructions in 2.1.1 and at step 2 - resuspend Dynabeads anti-Salmonella by vortexing until the pellet in the bottom of the tube disappears and aseptically add **20 µl** of properly mixed Dynabeads anti-Salmonella into sample tubes 1 and 2.

For the manual method, follow the instructions in 2.2.2 and at step 2 - resuspend Dynabeads anti-Salmonella by vortexing until the pellet in the bottom of the tube disappears and aseptically add **40 µl** of properly mixed Dynabeads anti-Salmonella into each eppendorf sample tube. Proceed as directed in each method. Steps 1-11 should be repeated on a five-fold dilution of the overnight incubated samples that returned presumptive Salmonella negative results after 6 h IMS analysis.

2.3 Specificity And Sensitivity

Dynabeads anti-Salmonella reacts with all current Salmonella serovars of importance as the cause of human and animal disease occurring in food, feed and environmental samples. This currently covers somatic groups from B-Z with variable reactivity depending on the serotype.

These protocols for using Dynabeads anti-Salmonella will determine the presence or absence of one viable Salmonella in 25 g of sample if this one cell is able to replicate and not out-competed by resident background flora during the overnight pre-enrichment. Using Dynabeads anti-Salmonella enables visible growth of Salmonella on a plating medium from a pre-enriched sample containing as low as 100 Salmonella/ml against a background of enteric competing flora greater or equal to 10^6 organisms/ml. Dynabeads anti-Salmonella significantly concentrates Salmonella from a mixed culture. For example, an initial ratio of Salmonella versus enteric 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 Salmonella in a mixed culture.

CONFIRMATION

Presumptive Salmonella colonies must be confirmed by standard biochemical and serological testing.

The accuracy of the method is not measurable since IMS is a qualitative, not a quantitative method. Several target bacteria may be bound to the beads and give rise to only one colony forming unit on the selective plating media. The precision is dependent on the extent to which particles are recovered from different sample matrices.

FALSE NEGATIVE/POSITIVE RATES

In seeded samples, Dynabeads anti-Salmonella records a false negative rate ranging between 5-15% depending on the inoculum, type, serovariant, background flora and sample matrix. In the same sample the conventional method ISO 6579 records a false negative rate of 5-25%. In naturally contaminated samples, Dynabeads anti-Salmonella will give a false negative rate ranging between 2.5-10%. In the same sample the conventional method ISO 6579 will give a false negative rate of 22.5-35%. Dynabeads anti-Salmonella decreases the false negative rate compared to the conventional method ISO 6579. False positive rates do not occur since the possibility to verify presumptive colonies is always applicable. However the method depends on the user following good laboratory practices and avoiding cross-contamination of samples.

2.4 Factors Affecting Product 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-Salmonella are fully dispersed by vigorous vortexing for at least 10 seconds before use.
- It is important that filtered pipette tips are used to transfer samples into the test tubes for both manual and automated IMS.
- In extremely fatty, viscous and/or particulate samples, a two to ten-fold dilution of the 24 h pre-enriched sample using the described wash buffer could be made prior to IMS analysis. Such a dilution will not limit detection of Salmonella but rather ensure that maximum beads are recovered.
- During bead-bacteria complex magnetic capture it is essential that gentle rocking of the Dynal MPC is continued. This prevents binding of low mass debris, which is magnetic or magnetisable
- For manual IMS the performance is solely dependent on the extent to which particles are recovered from different sample matrices.
- For 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 detect the presence of Salmonella in an otherwise positive sample.
- For automated IMS, to avoid cross-contamination of the prepared tubes, it is recommended that sample transfer into the tubes is performed in a designated area at least one metre from the prepared tubes. Sample tube-strips for the BeadRetriever are designed to fit into the rack in one direction only. Tip combs and tube tray should be inserted as instructed until a click sound is heard. At the end of the processing of a sample, remove the sample tray first before removing the tip combs. It is recommended that the tip combs remain for at least 10 minutes after the assay has been completed to allow for air-drying, before removal.

2.5 Precautions/Limitations

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

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

3 GENERAL INFORMATION

3.1 Storage/Stability

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

3.2 Technical Service

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

3.3 Warnings and Limitations

This kit is for research use only. Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide as a preservative, which is cytotoxic. Avoid pipetting by mouth!

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.

Certificate of Analysis (CoA) is available upon request.

Material Safety Data Sheet (MSDS) is available at www.invitrogen.com

3.4 Trademarks

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3.6 Warranty

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 Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

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4 REFERENCES

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AOAC - Samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the "PERFORMANCE TESTED" certificate number 970401.

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SPEC-06027