applied biosystems
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# Dynabeads® anti-Salmonella

**Catalog no. 71002** **Store at 2˚C to 8˚C**

**Rev. Date:** August 2012 (Rev. 009)

## Product Contents

| Product contents | Volume |
|---|---|
| Dynabeads® anti-Salmonella | 5 mL |

**Product capacity**
250 tests

Dynabeads® anti-Salmonella 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.

**Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

## Product Description

### 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™ benchtop instrument or performed using a manual method.

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.

### Intended User

Any user who is 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.

### 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 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, 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 hours 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 "Sample Preparation"). 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.

## Required Materials

**For performing automated IMS:**

- BeadRetriever™ instrument .
- BeadRetriever™ tubes and tips.

**For performing manual IMS:**

- Magnets: MPC™-6, MPC™-1, MPC™-S.
- Mixer allowing tilting and rotation of tubes (e.g. MX1, MX4, Sample Mixer).
- Micropipette (10–100 µL).
- 1-mL dispenser pipette.
- Stomacher apparatus and stomacher bag with filter.
- Test tubes, glassware, loops, swabs.
- Washing buffer (PBS Tween®): 0.15 M NaCl, 0.01 M Sodium-phosphate buffer, pH 7.4, with 0.05% Tween®-20. (Autoclavable at 121°C for 15 min.)
- Pre-enrichment broths such as buffered peptone water (BPW).
- Enrichment and selective culture media.

## General Guidelines

- Carefully read the instrument operating instructions of the BeadRetriever™ before use.
- To avoid cross-contamination and for safety reasons, perfom immunomagnetic separation 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.

## Protocol

### Prepare Sample

- Weigh 25 g of sample material and place into a stomacher bag with filter and add 225 mL of preenrichment 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, (e.g. bony meat, pasta, etc.) a blender is preferred prior to using a stomacher bag with filters to avoid the risk of perforation.
- For environmental samples using a swab, place the swab into 10–50 mL of pre-enrichment broth.
- Incubate the sample in the stomacher bag for 18–24 hours at 37°C.
- Mix the pre-enriched sample thoroughly by homogenizing once more. Pipet 1 mL aliquot of the filtered suspension for the immunomagnetic separation procedure. Change to a new pipette tip for each new sample.

### Method For Shell Eggs

1. Wash dirty eggs with a stiff brush under running water, and dry with a paper towel.
2. Dip the eggs into 70% ethanol for 5–10 sec and allow to dry. Alternatively follow any standard procedure of disinfecting shell eggs.
3. Aseptically crack open the eggs and mix/blend thoroughly both white and yolk.
4. Add ferrous sulphate ($FeSO_4$) solution to a final concentration of 35 mg/L, and pre-incubate the egg mixture at 37°C for 6 hours.
5. Mix the egg mixture thoroughly, and 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.
6. Re-incubate the remaining undiluted egg mixture overnight at 37°C.

### Automated Immunomagnetic Separation

**Automated Immunomagnetic Separation 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 labeled sample tube strip from the sample rack and place in a second sample rack (one meter 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 according to the "Post IMS" section.
13. Remove the sample tip combs and discard into a biohazard waste container together with the tube strips.

### Manual Immunomagnetic Separation

1. Remove the magnetic plate and load one 1.5-mL Eppendorf tube for each sample into the MPC™-S.
2. Resuspend Dynabeads® anti-Salmonella by vortexing until the pellet in the bottom of the tube disappears and pipet 20 µL into each tube.
3. Add 1 mL of the pre-enriched filtered sample aliquot and close the tube. Change pipette tip for each new sample.
4. Invert the MPC™-S rack five times to mix sample and beads. Incubate at room temperature for 10 min with gentle continuous agitation to prevent the beads from settling.

5. Insert the magnetic plate into the MPC™-S. Allow 3 min for proper recovery of beads. During this period, invert the rack several times to concentrate the beads into a pellet on the side of the tube.
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 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 once.
10. Repeat steps 5–7 once.
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 according to the "Post IMS" section.

## 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**

The rapid method is recommended for processed or foods known to harbor 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.).

**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 the "Automated Immunomagnetic Separation", section, 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 "Immunomagnetic Separation – manual IMS", 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 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 hours IMS analysis.

## 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 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.

## 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 little as 100 *Salmonella*/mL against a background of enteric competing flora ≥ $10^6$ 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.

## False Negative/Positive Rates

Dynabeads® anti-Salmonella decreases the false negative rate compared to the conventional method ISO 6579, as described in Table 1.

Table 1: False negative rate using Dynabeads® anti-Salmonella vs. the more conventional method ISO 6579

| Sample | False negative rates using Dynabeads® anti-Salmonella | False negative rates using ISO 6579 |
|---|---|---|
| Seeded samples | 5–15% | 5–25% |
| Naturally contaminated samples | 2.5–10% | 2.5–35% |

False positive rates do not occur since the possibility to verify presumptive colonies is always applicable.

## Factors Affecting Product Performance

- Perform the IMS procedure on a benchtop instrument at room temperature, and use room temperature reagents.
- Ensure that the Dynabeads® anti-Salmonella are fully dispersed by vortexing >10 sec 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-hour 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 with continous rocking of the MPC™ to prevents binding of magnetic or magnetizable low-mass debris.
- For manual IMS the performance is solely dependent on the extent to which particles are recovered from different sample matrices.
- Make sure not to aspirate and discard the isolated bead-bacteria complexes during manual IMS. Failure to recover the bead-bacteria complexes results in failure to detect the presence of *Salmonella* in an otherwise positive sample.
- To avoid cross-contamination of the prepared tubes in automated IMS, transfer the sample into the tubes in a designated area at least one meter from the prepared tubes.
- Sample tube-strips for the BeadRetriever™ are designed to fit into the rack in only one direction.
- 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 min after the assay has been completed to allow for air-drying before removal.

## Description of Materials

Dynabeads® anti-Salmonella are uniform, superparamagnetic, polystyrene 2.8 µm beads with affinity-purified antibodies against *Salmonella* covalently bound to the surface.

## Related Products

| Product | Cat. no. |
|---|---|
| MPC™-1 | 12001D |
| MPC™-6 | 12002D |
| MPC™-S | A13346 |
| MX1 | 15907 |
| MX4 | 61506 |
| Sample Mixer | 94701 |
| BeadRetriever™ | 15950 |
| BeadRetriever™ Tubes and Tips | 15951 |

REF on labels is the symbol for catalog number.

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