



[Food testing reagent]

NH BEADS O26 << Instruction Manual >>

*Read this manual before you start using this reagent

[Introduction]

The detection and isolation of *E.coli* O26 from food products have been difficult only by the culture of selective agar for *E.coli* O26, because a lot of competing flora present in food products.

This reagent using immune magnetic beads can be concentrate and isolate *E.coli* O26 from food products on the selective agar efficiently.

[Kit contents]

A: Magnetic beads labeled anti- <i>E.coli</i> O26 antibody solution	500 μ L \times 1 tube (25 tests)
B: Instruction manual	1 sheet

[Additionally required materials and instruments]

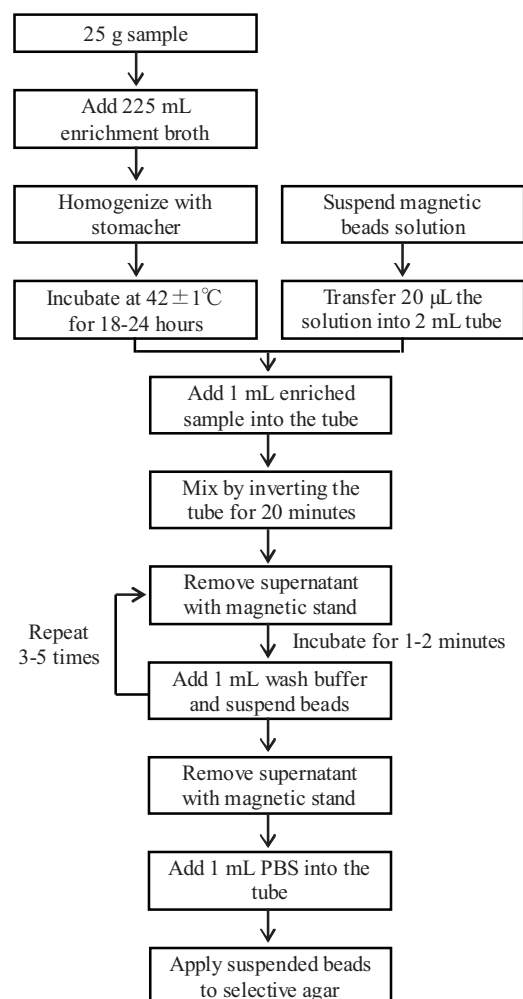
- Enrichment broth (e.g. mEC with novobiocin, mEC, mTSB)
- Incubator ($42 \pm 1^\circ\text{C}$)
- Sterilized 2 mL centrifuge tubes
- Sterilized PBS
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips
- Magnetic stands for centrifuge tubes (capable of testing more than 3500 gauss)
- Selective agar (preferably using both CT-RMAC and chromogenic substrate agar)
- Stomacher and stomacher bags (preferably with a filter)
- Balance (capable of weighting 25 g)
- Sterilized wash buffer (PBS+0.05% Tween20)
- Autoclave

[Test procedures]

- (1) Weigh 25 g samples into the stomacher bag and add 225 mL enrichment broth.
- (2) Homogenize with a stomacher for 1 minute.
- (3) Incubate the homogenized sample at $42 \pm 1^\circ\text{C}$ for 18-24 hours.
- (4) Remove the stomacher bag from the incubator and mix the culture gently using side-to-side motion.
- (5) Transfer 1 mL enriched sample into a sterilized 2 mL centrifuge tube added 20 μ L magnetic beads solution with a sterilized pipette (Note 1).
- (6) Mix by inverting magnetic beads solution and sample solution generally for 20 minutes at room temperature using e.g. rotator.
- (7) Incubate the tube on the magnetic stand for 1-2 minutes at room temperature.
- (8) Remove supernatant using sterilized pipette with setting the tube in the magnetic stand (NOTE 2).
- (9) Add 1 mL wash buffer and suspend the magnetic beads without setting the tube in the magnetic stand.
- (10) Repeat 3-5 times of above procedure of (8)-(9) to wash the magnetic beads.
- (11) After remove the supernatant completely, add 100 μ L PBS into the tube and suspend magnetic beads without setting the tube in the magnetic stand.
- (12) Apply suspended magnetic beads to two selective agars (preferably using both rhamnose MacConkey agar with cefixime and tellurite (CT-RMAC) and chromogenic substrate agar).

Note 1: Before transfer magnetic beads solution, suspend the solution so that precipitate does not remain at the bottom of the tube.

Note 2: When remove supernatant from the tube, be careful not to aspirate magnetic beads.



[Efficiency of this kit]

When standard strain of *E.coli* O26 concentrated with this reagent culture on a standard agar, the number of colonies on the standard agar shows more than 90% of recoveries.

[Precautions]

Precautions in using this reagent

- Use this reagent in accordance with the procedure described in this manual.
- Do not use expired reagents. The expiration date is indicated on the package.
- This reagent is intended to use for concentration of *E.coli* O26 from various food products. Not intended for diagnostic use.
- Do not use the mixture reagents of varying the manufacturing number.
- Confirm in each manufacture or distributor about instruments and reagents that used in this procedure.
- NH Foods Ltd. makes no warranty, whether expressed or implied. Each user takes responsibility for the judgment and the use of the results provided using this products.
- Each user is expected to verify the appropriateness of each operative procedure and the application to individual food materials.
- NH Foods Ltd. will not be liable for any damages, including special or consequential damages, or expense arising directly or indirectly from the use of this products.
- NH Foods Ltd. may end the production or change the specifications of this reagent without notice.

Precaution in the prevention of hazard

- Because *E.coli* O26 has been shown to have low infective dose, this reagent is intended for use by personnel familiar with the appropriate aseptic techniques.
- Extreme care should be taken in handling samples and enrichment cultures, and use personal protective equipment such as protective gloves, safety glasses.
- When the materials can cause the infection such as samples contact eye or mouth, immediately provide first aid such as flush eyes with running water or rinse mouth with plenty water and consult a physician.
- When there are disorders such as diarrhea and pyrexia after using this reagent, immediately consult a physician.

Precaution in the disposal

- Decontaminate used materials and instruments in this procedure such as tubes, pipettes, enriched sample, cultured selective agar, and removed supernatant by autoclave, etc.
- Any disposal practice in compliance with country, local state, and federal laws and regulations.

[Storage and expiration date]

- Storage: This reagent should be stored at 2-8°C and must not be frozen.
- Expiration date: 24 months from the date of manufacture.

[References]

- (1) MHLW Notification No.1120 “Inspection Method for Intestinal hemorrhagic *E.coli* O26, O103, O111, O121, O145 and O157”



COSMO BIO CO., LTD.

Inspiration for Life Science

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN

URL: <http://www.cosmobio.co.jp>

e-mail: export@cosmobio.co.jp

[Outside Japan] Phone : +81-3-5632-9617

[国内連絡先] Phone : +81-3-5632-9610

FAX : +81-3-5632-9618

FAX : +81-3-5632-9619