Captivate™ IMS Beads

CAP001	Captivate™ O157
CAP003	Captivate™ O26
CAP004	Captivate™ O111
CAP005	Captivate™ O103
CAP006	Captivate™ O145
CAP007	Captivate™ O104
CAP008	Captivate™ O121
CAP010	Captivate™ O91

PLEASE NOTE – each bead within the Captivate range is specific to a particular *E. coli* O group, therefore if testing for more than one O group the appropriate tests need to be run in parallel. All follow the same format and protocol outlined in this document.

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Intended Use

Captivate is a range of immunomagnetic separation (IMS) beads, coated with specific antibody, intended for the concentration and isolation of specific *Escherichia coli* O groups from food, animal feed, beverages, pharmaceutical or environmental samples. The use of IMS is outlined in USDA MLG, ISO 16654:2001 + A1:2017, and ISO 13136:2012. Captivate beads are not intended for use in the diagnosis of disease or other conditions in humans.

Description

Since *E. coli* O157:H7 was first identified, shiga-toxin producing *Escherichia coli* (STEC, also referred to as VTEC - verocytotoxin-producing *E. coli*) of various serotypes have become an increasing concern for public health. Numerous outbreaks have been attributed to STEC serotypes and symptoms may include bloody or acute diarrhea, and/or the development in some patients of Haemolytic Ureamic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP).

Non-O157 VTEC are associated with the following: fresh meat; ready-to-eat fermented meats (e.g., salami, pepperoni); fresh produce (ready-to-eat pre-cut vegetables and sprouted seeds); raw and low heat-treated milk and derived dairy products; and in the hides and fleeces of cattle, sheep and goats.

Captivate beads are antibody coated paramagnetic particles for the specific immunomagnetic separation of microorganisms. This patented technology consists of microscopic paramagnetic particles with a magnetite core and a "ceramic" zirconium oxide coating. The beads are manufactured by a high-speed blending process and typically have a diameter range of 1-4µm, with an average size of 2.5µm. Antibodies, purified to surface components of the target microorganism, are covalently coupled to the bead. With careful antibody selection, a highly specific separation system for microorganisms is produced.

The pre-coated beads are designed for the IMS of target bacteria from enrichment cultures. A sample is taken from a filter stomacher bag and incubated with the Captivate beads for 30 minutes. The bead/microorganism complexes are then removed from the sample by placing the sample in a magnetic concentrator device. This separates them from the background organisms and interfering materials. The complexes are then washed using a PBS/Tween® 20 wash buffer to remove non-specifically bound material and the beads can be plated out onto an appropriate selective agar and incubated as required. CAP001 can be paired with SMAC (NCM0167) supplemented with NCM4045, or the chromogenic SMAC-BCIG (NCM1007) for improved specificity, supplemented with NCM4045.Other Captivate products can be paired with generic *E. coli* agar such as TBX (NCM1001)

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The IMS technique will increase the sensitivity of the methodology, and in most circumstances, results can be achieved 24 hours earlier than standard protocols. These products can also serve as a capture system for rapid detection systems.

Specification

Working concentration:	Typically 5mg/mL
Antibody:	Particles coated with high avidity, high affinity purified and absorbed polyclonal antibodies to cell surface antigens
Specificity:	Reacts with target organism
Average size:	2.5µm (typical range 1-8µm)
Storage:	2-8°C (may be shipped at ambient temperature)
Shelf life:	2 years
Formulation:	Particles are suspended in: Phosphate Buffered Saline (PBS) Bovine Serum Albumin (BSA) 1% Sodium azide 0.09%

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Test Procedure

1. Weigh 25g of sample into a stomacher bag then add 225ml of enrichment broth and homogenize. Larger sample weights can be used but the enrichment broth volume must be adjusted accordingly.
2. Incubate for 6 hours at 42°C.
3. Homogenize the enrichment culture then allow to settle for 5 minutes.
4. Mix the Captivate beads well and add 20µl Captivate beads to a suitable tube (able to take approximately 1.5-2.5ml volume).
5. Add 1ml of the enrichment culture (taking care to avoid the pipetting of food debris) to the tube containing the Captivate beads.
6. Cap the tube tightly and rota-mix the suspension at room temperature for 30 minutes.
7. Insert the tube into a magnetic separator for 3 minutes to separate the particles.
8. Carefully aspirate the supernatant from the tube without removing the particles and replace cap.
9. Remove the magnet from the rack (or remove tubes from the magnetic separator rack) and add 1ml of wash buffer. Resuspend the particles by inverting several times.
10. Wash the particles twice more by repeating steps 7-9. Finally resuspend in 100µl of wash.
11. Remove the complexed resuspended particles and transfer onto an appropriate selective agar. Streak/spread the initial inoculum to achieve single colonies.
12. Incubate the plates appropriately and check for growth of typical *E. coli* colonies.
13. Suspect colonies should be confirmed using biochemical or serological methods.

The Captivate Separator Rack (CAP100-12P) is commonly used as a magnetic separation device. Automated systems are available on the market and can also be used.

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Wash buffer formulation and preparation instructions

Phosphate Buffered Saline plus Tween®

Material	g/Liter
Sodium Chloride	8.0
Potassium Chloride	0.020
Disodium Hydrogen Phosphate	1.15
Potassium Dihydrogen Phosphate	0.2
Tween® 20 (Polysorbate 20)	0.5

pH: 7.0 ± 0.2 @ 25°C

1. Weigh components into a clean dry vessel and dissolve in RO water.
   Note – commercial (tablet) preparations of phosphate buffered saline may be used.
2. Sterilize by autoclaving at 121°C for 15 minutes or filter sterilize using a 0.2µm membrane filter.
3. Store solution for a maximum of 6 months and discard if contamination is suspected.

Quality Control Specifications

Appearance: Clear liquid with black pellet or black liquid when suspended.

Minimum QC Organisms

Captivate Beads	Minimum QC Strains
CAP001 (*E. coli* O157)	*Escherichia coli* NCTC 12900 (*E. coli* O157) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP003 (*E. coli* O26)	*Escherichia coli* NCTC 8783 (*E. coli* O26) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP004 (*E. coli* O111)	*Escherichia coli* NCTC 9111 (*E. coli* O111) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP005 (*E. coli* O103)	*Escherichia coli* NCTC 9103 (*E. coli* O103) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP006 (*E. coli* O145)	*Escherichia coli* NCTC 10279 (*E. coli* O145) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP007 (*E. coli* O104)	*Escherichia coli* NCTC 9104 (*E. coli* O104:H4) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP008 (*E. coli* O121)	*Escherichia coli* NCTC 9121 (*E. coli* O121) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)
CAP010 (*E. coli* O91)	*Escherichia coli* NCTC 9045 (*E. coli* O45) *Escherichia coli* NCTC 9001 (*E. coli* O1:K1)

Product Limitations

This product will bind the target *E. coli* O group strain and may cross react with Enterobacteriaceae expressing similar O antigens. Captivate beads are able to bind low numbers of the target *E. coli* O group in a high background of competing flora.

Precautions

- Care should be taken to prevent splashing or aerosol formation and all work should be performed in an appropriate safety cabinet.
- Wash buffer must be disinfected prior to disposal as it may be contaminated.

- Sodium azide (0.09%) is used as a preservative. Appropriate precautions must be taken for disposal of this material.

Storage
Store at 2 – 8°C away from direct sunlight.

References

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7. Vernozy-Rozand, C. (1997). Detection of *Escherichia coli* O157:H7 and other verotoxin- producing *E.coli* (VTEC) in food. Journal of Applied Microbiology 82, 537-551.
8. Wright, D.J., P.A.Chapman, and C.A. Siddons (1994). Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. Epidemiology and Infection 113, 31-39.
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10. USDA MLG 5B.04 2013 Laboratory Guidebook, Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges.
11. ISO 13136:2012 Microbiology of food and animal feed — Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups
12. ISO 16654:2001 + A1:2017 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157.

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