Captivate™

***Captivate*™** is a range of antibody coated paramagnetic particles for the specific **I**mmuno**M**agnetic**S**eparation **(IMS)** of microorganisms.

This patented technology consists of microscopic paramagnetic particles. The beads have a magnetite core and a "ceramic" zirconium oxide coating. The beads are manufactured by a high speed blending process and typically cover a size diameter range of 1-4 µm, with a 2.5µm average size.

Purified antibodies 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. The beads can then be plated out onto the appropriate selective agar media and incubated as described.

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.

Special Notes on IMS Techniques.

There are important factors that affect the performance of **IMS** techniques. Thorough mixing of the particles and sample allied with efficient recovery of the beads from the sample matrix is paramount to the success of this technique. Care must be taken not to aspirate the sample vigorously as this can result in the loss of captured target organisms. Certain sample types (e.g. very fatty, particulate and viscous samples) can interfere with bead recovery. To counteract this interference, samples can also be diluted in PBS-Tween® e.g. 1:2-1:4, reducing the effect of the matrix and allowing more efficient bead recovery. Alternatively with problem samples, after the initial magnetic separation the incomplete removal of the sample (i.e. remove 800µl) and continuation of the wash protocol as described can minimise bead losses.

***Captivate*™ Product Specification**

Working concentration:	Typically 5mg/ml
Fe3O4 content:	29-33% w/w
Antibody:	Particles coated with high avidity, affinity purified and absorbed polyclonal antibodies to cell surface antigens.
Specificity:	Reacts with target organism.
Average size:	2.5 µm (typical range 1-8µm)
Formulation:	Particles are suspended in PBS plus1% BSA pH 7.3-7.5 and 0.09% azide as preservative.
Storage:	8°C (may be shipped at ambient)
Shelf life:	2 years.

Captivate™ O157

CAP 1

Description
Captivate™ O157 are magnetisable particles coated with specific antibody intended for the isolation of *E. coli* O157:H7 from food, animal feeds, beverages, pharmaceutical or environmental samples. The particles help to concentrate O157:H7 cells in mixed culture reducing the probability of missing low numbers or overgrowth of O157:H7 colonies by competing flora. In fact, immunomagnetic separation is now regarded as the gold standard method for isolation of *E. coli* O157:H7 from food and environmental samples.

E. coli O157:H7 is the primary serovar associated with food borne gastrointestinal infection, resulting in self-limiting diarrhoea, that can lead to serious disease conditions such as haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

The organism itself is associated with raw meats and unpasteurised milk1, probably due to the implication of farm animals and particularly cattle as carriers of *E. coli* O157:H7. Large outbreaks have been recorded in the United States from consumption of unpasteurised apple juice (apple cider) possibly as a result of using apples which have fallen to the ground where the potential for contamination with the organism exists[3,4].

Enrichment Protocol for *E. coli* O157:H7.
The recommended protocol for the isolation of *E. coli* O157:H7 employs a 6 hour enrichment step at 42°C in modified Tryptone Soy Broth (mTSB, LAB 165) plus novobiocin (X150) followed by **IMS** (see below) and plating onto Sorbitol MacConkey Agar (LAB 161 or HAL006) supplemented with or without the addition of cefixime and potassium tellurite (X161)[5-8]. It is also recommended that a further **IMS** and inoculation of SMAC plates is performed after incubation of the sample for 24 hours. Alternative enrichment protocols using different media have been described e.g. Buffered Peptone Water (LAB 46) plus VCC (X546)[5-8].

Generic *Captivate™* IMS Procedure
1) Add 20µl of well mixed ***Captivate™*** particles to a suitable micro-tube (1.5 - 2.5ml volume).
2) To this add 1ml of the enrichment culture taking care to avoid transfer of sample debris.
3) Cap tube tightly and rotamix the suspension for 30 minutes at room temperature.
4) Insert tube into magnetic separator rack for 3 minutes to concentrate the beads to a pellet. Gently invert the rack several times to aid pelleting of the beads.
5) Carefully aspirate the supernatant from the tube and cap without removing particles, taking care to avoid splashing.
6) Remove magnet from rack or tubes from the rack and add 1ml of wash. Cap and resuspend particles by inverting several times.
7) Repeat separation and wash steps 4-6 twice more. Finally resuspend particles in 100µl of wash.
8) Remove 50µl of the complexed, resuspended particles to the plating media, streaking for single colonies. Incubate plates at 37°C for 18-24 hours and examine for typical colonies.

Phosphate Buffered Saline plus Tween®.

Formula	g/litre
Sodium chloride	8.0
Potassium chloride	0.020
Disodium hydrogen phosphate	1.15
Potassium dihydrogen phosphate	0.2
Tween® 20.	0.5

pH: 7.0 ± 0.2

Dissolve the components in deionised water and check the pH. Sterilise the solution by autoclaving at 121°C for 15 min. Allow the solution to cool and check the pH. Store in the dark and use within one month.

Interpretation: Examine the SMAC and CTSMAC plates for typical *E. coli* O157 non-sorbitol fermenting colonies that are smooth and circular, 1-3 mm in diameter that are colourless to pale orange. Confirm the colony identity with commercially available latex agglutination kits or antisera.

Product Presentation
Captivate™ O157 is available in packs of 50 test, product code CAP001-050 and 250 test, product code CAP001-250. Materials required, but not provided, include phosphate buffered saline-Tween® 20, pipettes and tips, stomacher machine and bags, magnetic separator rack and culture media. Magnetic separating racks (CAP-100-12P) and rotating mixers (CAP101-58) are also available from Lab M..

References

1) Padhye, N.V., and Doyle, M.P. (1992). *Escherichia coli* O157:H7: Epidemiology, Pathogenesis and Methods for Detection in Food. J.Food.Prot. **55**, 555-565.

2) Martin, M.L. *et al* (1986) Isolation of *Escherichia coli* from cattle associated with two cases of hemolytic syndrome. Lancet **ii** 1043.

3) Besser, R.E. *et al* (1993) An outbreak of diarrhoea and haemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA **259** 2217-2220

4) McCarthy, M. (1996) *E. coli* O157:H7 outbreak in USA traced to apple juice. Lancet **348** 1299.

5)Wright, D.J., Chapman, P.A. and Siddons, C.A. (1994). Immunomagnetic separation as a sensitive method for the isolation of *Escherichia coli* O157 from food samples. Epidemiology and Infection **113**, 31-39.

6) Bolton, F.J.; Crozier, L.; Williamson, J.K. (1995) New technical approaches to *Escherichia coli* O157. PHLS Microbiol. Dig. **12** 67-71.

7) Vernozy-Rozand, C. (1997). Detection of *Escherichia coli* O157 and other VTEC in food. Journal of Applied Microbiology. **82**, 537-551.

8) Ogden, I.D.; Hepburn, N.F.; & MacRae, M. (2001). The optimisation of media used in the immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods. J. Appl.Mic. 91, 373-379.

Captivate™ O26

CAP 3

Captivate™ O111

CAP 4

Captivate™ O103

CAP 5

Captivate™ O145

CAP 6

Description
There is now growing concern that VTEC's could be playing a more significant role in human disease than currently estimated. Current microbiological methods do not permit the sensitive isolation of non- O157-VTEC. Unlike *E. coli* O157:H7, which is sorbitol negative, this group does not appear to have any common distinct biochemical properties. Therefore using a screening isolation medium such as Sorbitol MacConkey Agar does not help in the isolation of these organisms.

To address this basic problem Lab M has developed a quartet of individual immunomagnetic separation reagents; O26, O103, O111 and O145 to aid the isolation of the other common serotypes of verotoxigenic *E. coli* (VTEC). Therefore, in combination with the ***Captivate™*** O157 reagent, the "top five" VTEC as identified by the World Health Organisation can be targeted with these reagents.The **IMS** step should greatly increase the chance of isolating these organisms.

Enrichment methods are currently being developed by researchers and consequently there are no standard protocols to recommend. Bearing this in mind, we suggest users try the same enrichment protocol that we recommend for *E. coli* O157 at 37°C and 42°C with a generic *E. coli* plating medium such as Tryptone Bile Glucuronide Agar (HAL 3) or TBA (LAB 72) plus MUG (MC406). SMAC-BCIG (HAL 6) has also been used for this application as it contains the glucuronide chromogen, which the majority of *E. coli* react with. Some workers have recommended the use of "Enterohaemolysin agar" sheep blood agar for detection of VTEC. Verocytotoxin production has been shown to be closely linked with the enterohaemolytic phenotype.

References
Bielaszewska, M. & Karch, H. (2000). Non-O157:H7 Shiga toxin (verocytotoxin)-producing *Escherichia coli* strains: epidemiological significance and microbiological diagnosis. World Journal of Microbiology and Biotechnology. Vol **16**, 8-9, 711-718.

Beutin, L., Montenegro, M.A., Orskov, I., Prada, J., Zimmerman, S. and Stephan, R. (1989). Close association of verocytotoxin (shigalike toxin) production and enterohaemolysin production in strains of *Esherichia coli*. Journal of Clinical Microbiology **27**, 2559-2564.

Captivate™ Salmonella

CAP 2

Description
This product is designed to capture and concentrate the common serotypes of *Salmonella* involved in human and animal disease from enriched samples. The particles are coated with affinity purified polyclonal antibody directed towards common somatic and flagellar antigens. This gives an excellent high avidity broad spectrum **IMS** reagent for the capture of salmonellas. Due to the large variation in *Salmonella* serotypes and antigen expression there is naturally strain dependent variation in the capture efficiency.

Custom Coating Service

A coating service is available for coating our **IMS** reagent with alternative antibodies. Prices will be calculated on an individual basis.

For more information please contact: Dr. D.S. Illingworth.

Tel: +44 (0)161 797 5729

Fax: +44 (0)161 762 9322

Email: simon.illingworth@labm.com