FluIDect. APPLICATION NOTE

Keywords: Bacterial detection, *E. coli* O157, functionalised µBeads, Colligo Mag µBeads

# Colligo Mag µBeads: functionalised magnetic µBeads for detection and concentration of *E. coli* O157

Anne Klabunde and Lorena Tuchscherr de Hauschopp

Correspondence: Email: application@fluidect.com

## Abstract

Rapid and reliable detection of pathogenic bacteria in food, water and environmental samples remains a critical challenge in public health and food safety, as conventional culture- and PCR-based methods are time-consuming and require specialised equipment. FluIDect® Colligo Mag µBeads (anti-*E. coli* O157 functionalised magnetic microbeads, µBeads) offer a fast, selective alternative, using immunomagnetic separation (IMS) to concentrate *E. coli* O157 directly from unenriched samples. Antibody-functionalised polystyrene µBeads bind *E. coli* O157 strains, and the resulting bead–bacteria complexes are isolated with a simple magnetic platform. After only 60 minutes of incubation, capture efficiencies of 76–83% were achieved between $10^2$ and $10^4$ CFU/mL, and efficient capture was maintained at concentrations as low as 10 CFU/mL (73.4%).

Once captured, bacteria bound to the µBeads can be read out by multiple detection modalities, including plating and flow cytometry with nucleic acid staining, allowing flexible integration into existing laboratory workflows. The platform demonstrated specific capture of *E. coli* O157 with low cross-reactivity against *Salmonella enterica* serovar Typhimurium (≤10.1%), corresponding to an approximately 8- to 12-fold preference for the intended target. Robust and reproducible capture performance was achieved across a range of sample matrices, including phosphate-buffered saline (PBS), deionised water, artificial urine, mud-spiked water and 1% milk (74.7–81.4%), while recovery in whey was reduced (51.7%) and partially restored by dilution (61.8% in 50% whey). Furthermore, the functionalised µBeads retained full capture activity for at least nine months of storage (data not shown). Overall, these findings underscore their suitability for routine, real-world diagnostic and monitoring applications.

## Introduction

Contamination of food and water supplies with pathogenic *Escherichia (E.) coli* O157:H7 represents a serious public health threat, associated with haemorrhagic colitis and haemolytic uraemic syndrome. Rapid and accurate detection of this pathogen is essential for timely intervention, yet traditional microbiological methods — including culture-based assays and PCR — typically require 24–72 hours to yield actionable results and demand specialised laboratory infrastructure. Immunological approaches such as ELISA offer

greater speed but are often constrained by multi-step protocols, limited sensitivity in complex or turbid matrices, and reduced adaptability across sample types.

µBead-based capture platforms offer a compelling alternative, combining the high surface-to-volume ratio of functionalised particles with the specificity of antibody or protein-ligand interactions to concentrate and present target bacteria for downstream detection. Once loaded, the µBeads are compatible with multiple readout modalities, including flow cytometry with nucleic acid staining or other µBead-compatible detection assays, allowing seamless integration into diverse laboratory workflows.

In this application note, we evaluate the performance of the functionalised Colligo Mag µBeads for the specific capture of *E. coli* O157 across a range of relevant sample matrices, including PBS, water, artificial urine, mud-spiked water, and 1% milk. Capture specificity is confirmed by the high selectivity of the µBeads for *E. coli* O157 over the closely related *Salmonella enterica* serovar Typhimurium.

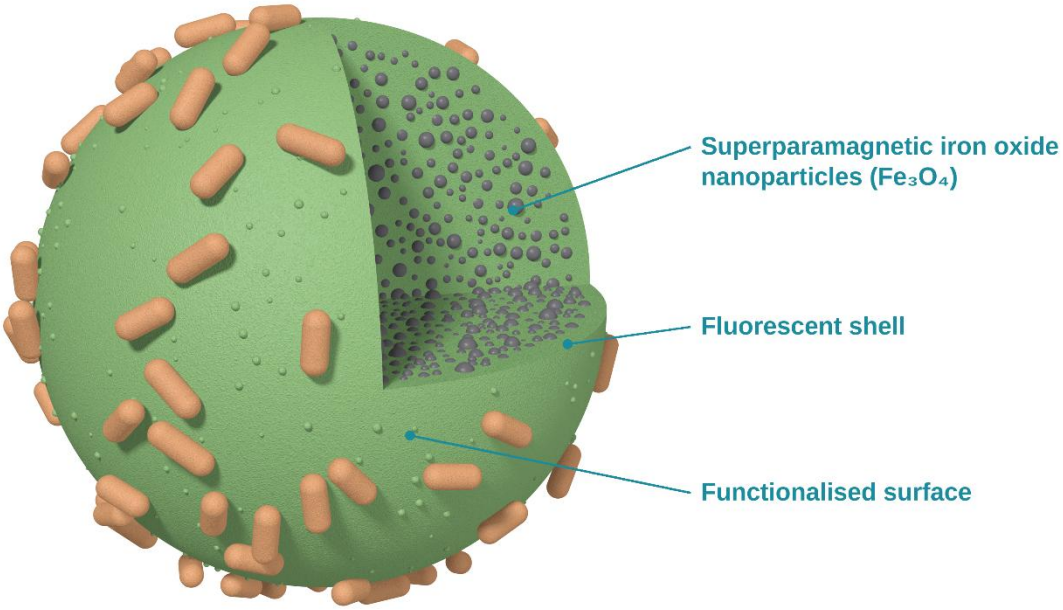



***Figure 1. Schematic of a fluorescent, superparamagnetic FluIDect Colligo Mag µBead*** *bearing captured E. coli bacteria on its surface. The cut-away section shows the polymer shell containing an embedded fluorescent dye and dispersed superparamagnetic iron oxide ($Fe_3O_4$) nanoparticles. These nanoparticles enable the bead to be separated using magnetic forces. The functionalised surface binds E. coli cells (shown in orange), enabling the bacteria to be selectively captured and concentrated from the sample. Schematic, not to scale.*

# Experimental Design

## Bacterial Capture & Detection

Colligo Mag µBeads were functionalised with antibodies specific for *E. coli* O157 using established in-house protocols. The functionalised µBeads were then incubated with suspensions of *E. coli* O157 or *Salmonella enterica* serovar Typhimurium prepared in the respective test matrix. Captured bacteria were recovered and plated on tryptic soy agar (TSA), and colonies were counted to determine CFU/mL.

## Design of the Experiment

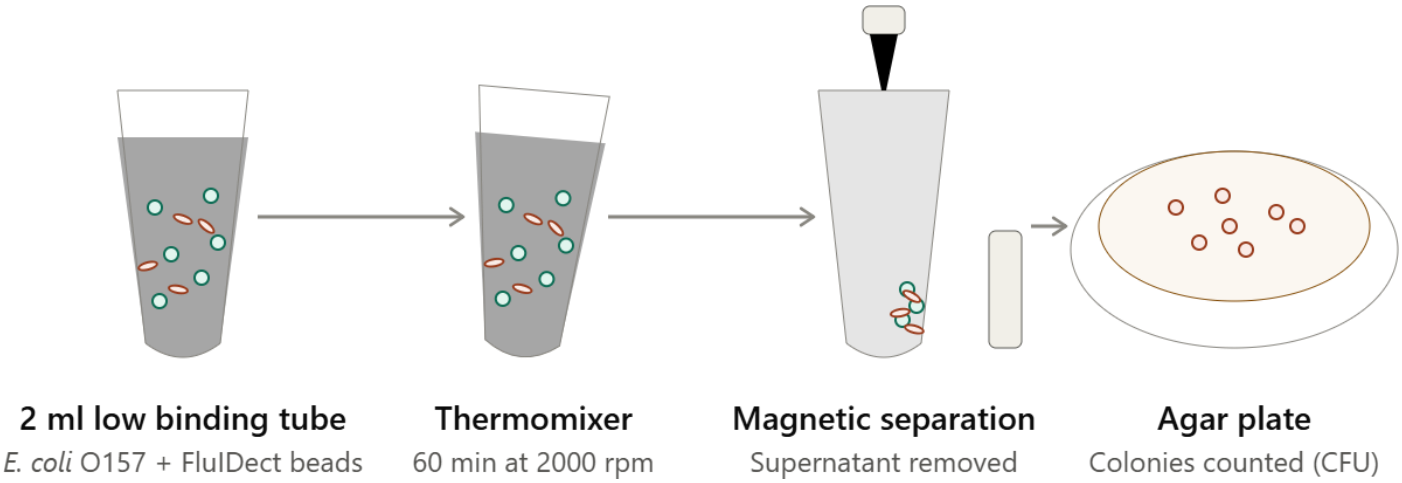



***Figure 2:*** *Workflow for the magnetic capture of E. coli O157 using FluIDect Colligo Mag µBeads. Combine E. coli O157 and Colligo Mag µBeads in a 2 ml low-binding tube. Incubate in a thermomixer for 60 minutes at 2000 rpm to allow the bacteria to bind to the bead surface. The Colligo Mag µBead–E. coli complexes were then pulled against the tube wall using a permanent magnet and the supernatant was removed. The captured bacteria were then plated on agar, and the number of colony-forming units (CFUs) was used to quantify the capture efficiency.*

## Assessment Conditions

- **Specificity**: anti-*E. coli* O157 µBeads challenged with *E. coli* O157 and *S. enterica* Typhimurium
- **Matrix robustness**: *E. coli* O157 (100 CFU/mL) spiked into PBS, deionised water, artificial urine, mud-spiked water, whey (100 and 50%) and 1% milk
- **Sensitivity**: *E. coli* O157 at $10^0$ to $10^4$ CFU/mL
- **pH tolerance**: PBS at pH 3, 5, 7.4 and 8.5
- **Protocol optimisation**: sample volumes of 300 µL to 1 mL and incubation times of 15 to 60 minutes

# Results

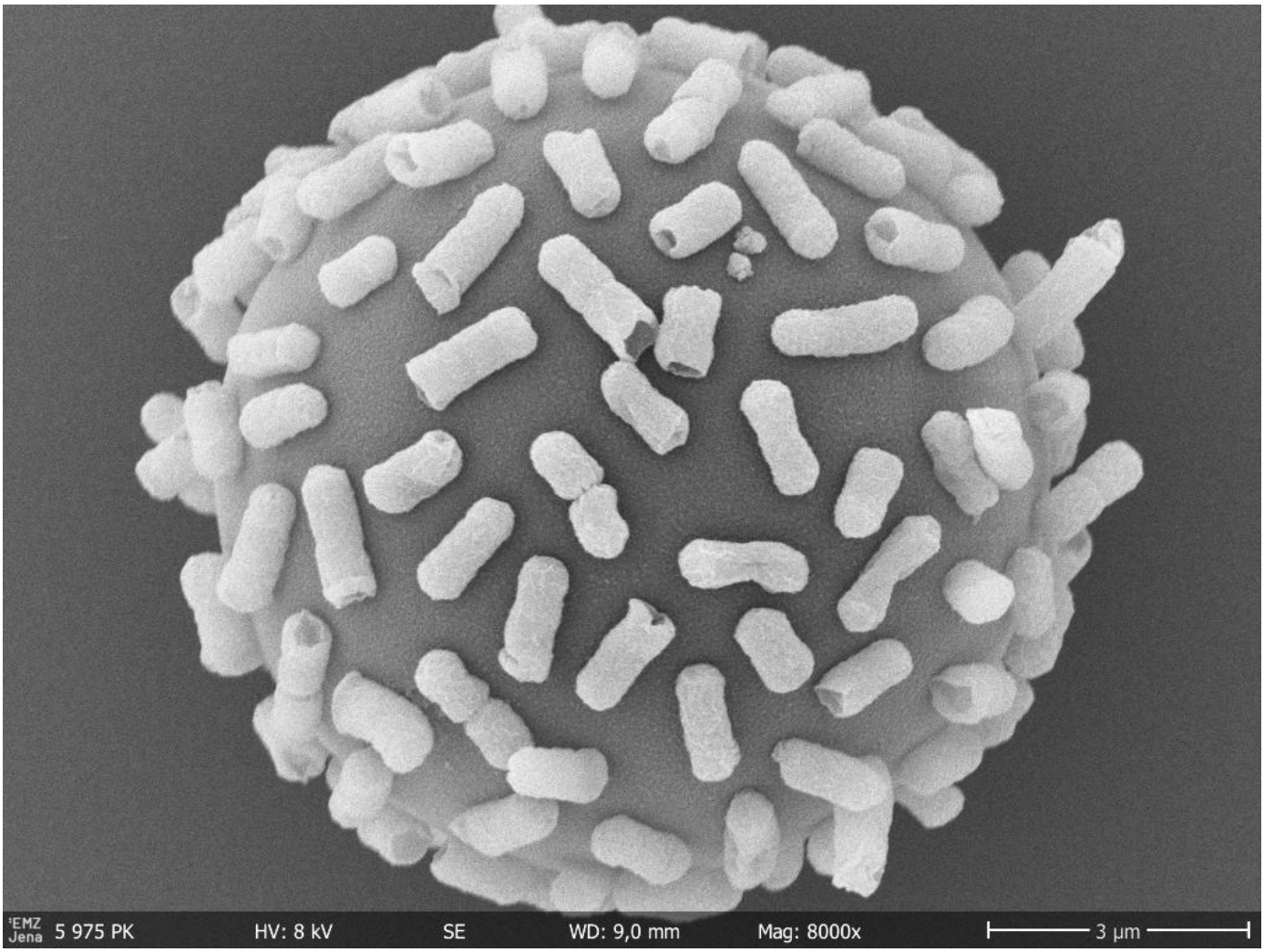



***Figure 3. Scanning electron micrograph of a FluIDect Colligo Mag µBead with captured E.*** *coli O157. Numerous rod-shaped bacteria are attached across the functionalised bead surface, showing efficient binding of the target organism. Imaging conditions: secondary electron (SE) detector, 8 kV accelerating voltage, 9.0 mm working distance, 8000× magnification. Scale bar: 3 µm.*

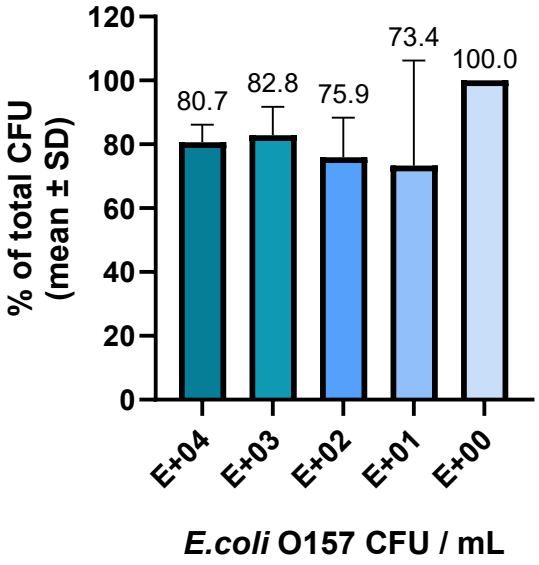



***Figure 4. Capture efficiency of FluIDect Colligo Mag µBeads (anti-E. coli O157) across a range of bacterial concentrations****. Antibody-functionalised µBeads were incubated with E. coli O157 at five concentrations spanning $10^4$ to $10^0$ CFU/mL. Capture efficiency, expressed as the percentage of total CFU recovered on the beads (mean ± SD, n = 6), remained high and consistent between $10^4$ and $10^2$ CFU/mL, reaching 80.7% at $10^4$ CFU/mL, 82.8% at $10^3$ CFU/mL and 75.9% at $10^2$ CFU/mL. At $10^1$ CFU/mL, mean capture efficiency was 73.4%, with markedly greater variability between replicates (larger SD), consistent with the increased stochastic effects expected when working near single-digit CFU inputs. At the lowest concentration tested ($10^0$ CFU/mL), all recovered CFU were bead-associated (100%); given the very low number of cells present at this input, this value should be interpreted with caution. These results demonstrate that the FluIDect Colligo Mag µBeads (anti-E. coli O157) maintain robust, high-efficiency capture (>75%) down to at least $10^2$ CFU/mL, and continue to capture E. coli O157 at inputs as low as $10^1$–$10^0$ CFU/mL.*

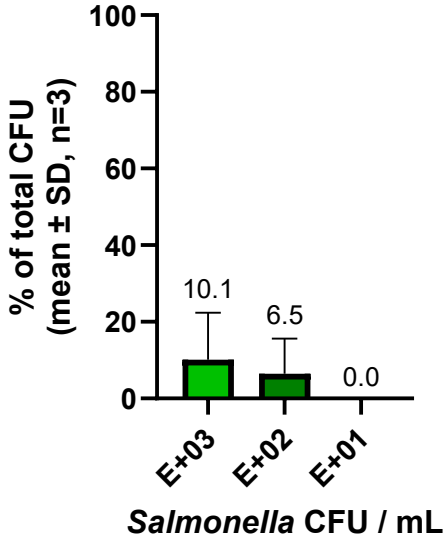



***Figure 5. Cross-reactivity assessment against Salmonella enterica serovar Typhimurium.*** *o confirm target specificity, FluIDect Colligo Mag µBeads (anti-E. coli O157) were challenged with Salmonella enterica serovar Typhimurium, a Gram-negative enteric bacterium closely related to E. coli at the genomic level (~84% DNA sequence identity between orthologous genes; McClelland et al., 2000) and of comparable size and morphology. Despite this similarity, cross-reactivity remained low across the concentration range tested (mean ± SD, n = 3). At $10^3$ CFU/mL, only 10.1% of the total Salmonella population was non-specifically captured, decreasing further to 6.5% at $10^2$ CFU/mL, with no capture detected (0.0%) at $10^1$ CFU/mL. These values contrast markedly with the 82.8% and 75.9% capture efficiency observed for E. coli O157 at the equivalent $10^3$ and $10^2$ CFU/mL concentrations, respectively (Figure 4), corresponding to an approximately*

*8- to 12-fold higher capture rate for the intended target over Salmonella. At $10^1$ CFU/mL, E. coli O157 was captured with a mean efficiency of 73.4%, whereas no Salmonella was recovered. Taken together, these results confirm that the antibody-functionalised µBeads retain high target selectivity, even against a genomically similar, morphologically comparable organism.*

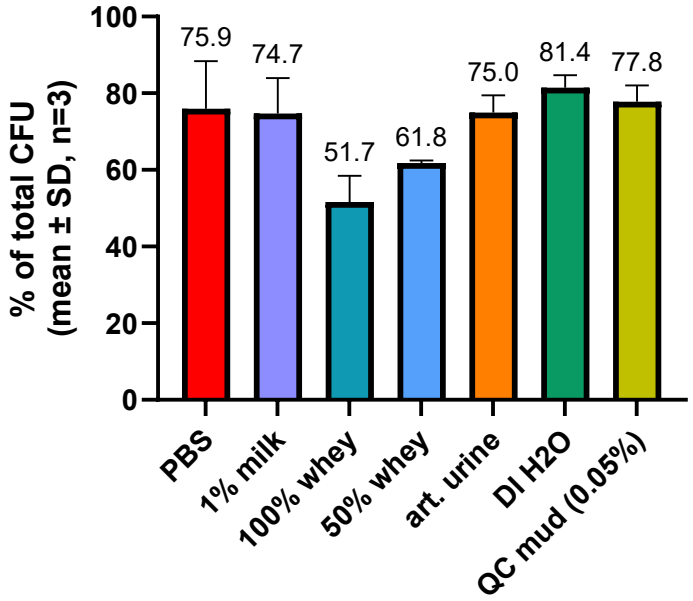



***Figure 6. Capture efficiency of FluIDect Colligo Mag µBeads (anti-E. coli O157) across diverse sample matrices.*** *E. coli O157 was added at a concentration of 100 CFU/mL to a range of sample types and captured using antibody-functionalised magnetic µBeads under standard assay conditions. The bacteria captured on the beads were then quantified by plating (mean ± SD, n = 3). A mean recovery of 75.9% was obtained in PBS, which served as the reference condition. Comparable performance was achieved in 1% milk (74.7%) and artificial urine (75.0%), while deionised water yielded the highest recovery (81.4%) and the turbid, mud-spiked water matrix (0.05%) also performed well (77.8%). Recovery was reduced in undiluted whey (51.7%) but improved to 61.8% when whey was diluted to 50%, indicating a matrix effect in this protein-rich sample that can be partially mitigated by dilution. These results demonstrate that Colligo Mag µBeads robustly and reproducibly capture E. coli O157 in a wide range of sample compositions, including complex and turbid matrices, with moderately reduced but still substantial recovery in whey. This supports their application in food safety, water quality and environmental monitoring workflows.*

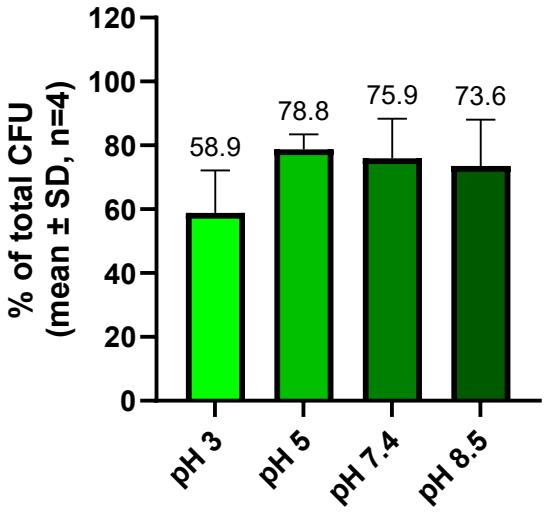



***Figure 7. Effect of sample pH on the capture of E. coli O157 by FluIDect Colligo Mag µBeads.*** *To assess the pH tolerance of the assay, E. coli O157 was added to PBS with a pH of 3, 5, 7.4 or 8.5. The bacteria were then captured using antibody-functionalised magnetic µBeads under standard assay conditions and the recovered bacteria were quantified by plating. Capture efficiency remained high across the entire tested pH range, with mean recoveries of 67.7% at pH 3, 82.4%*

*at pH 5, 76.8% at pH 7.4, and 69.7% at pH 8.5 (mean ± SD, n = 2). Although optimal performance was observed under mildly acidic to neutral conditions (pH 5–7.4), recovery at the extremes of the range remained above 65%. Overlapping standard deviations indicate that there was no substantial loss of antibody function or bead–bacteria binding. These results demonstrate that the µBeads tolerate a broad pH range, enabling their use with acidic and alkaline samples without the need for prior pH adjustment.*

## Protocol Optimisation: Sample Volume and Incubation Time

The standard assay conditions established in this study comprise combining 300 µL of the sample with 2 µL of FluIDect Colligo Mag µBeads and incubating the mixture at 2000 rpm in a thermomixer for 60 minutes. These conditions were selected following the systematic optimisation of both parameters. The protocol is flexible with respect to sample volume and can be scaled up to at least 1 mL without a substantial loss of capture efficiency. Larger volumes were not evaluated here as the maximum capacity of the thermomixer tube adapter used is 1.5 mL (see Figure 8). Shorter incubation times were also tested, but 60 minutes was found to be optimal, providing the highest and most consistent recovery. This incubation time was validated across all sample volumes tested, confirming that a single set of conditions can be applied throughout the working volume range (see Figure 9).

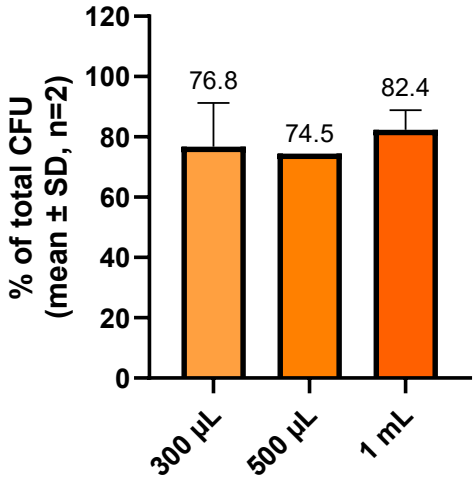



***Figure 8. Effect of sample volume on the capture of E. coli O157 by FluIDect Colligo Mag µBeads.*** *E. coli O157 was added to increasing volumes of PBS (300 µL, 500 µL, and 1 mL) and processed with antibody-functionalised magnetic µBeads under standard assay conditions. The recovered bacteria were then quantified by plating. Capture efficiency remained consistently high across the tested range, with mean recoveries of 76.8%, 74.5% and 82.4% at 300 µL, 500 µL and 1 mL, respectively (mean ± SD, n = 2). This indicates that the bead-to-sample ratio and magnetic separation are effective throughout this range. Volumes above 1 mL were not evaluated as the maximum capacity of the thermomixer tube adapter used is 1.5 mL, and mixing efficiency at this limit cannot be guaranteed. These results confirm reliable capture performance for sample volumes of up to 1 mL.*

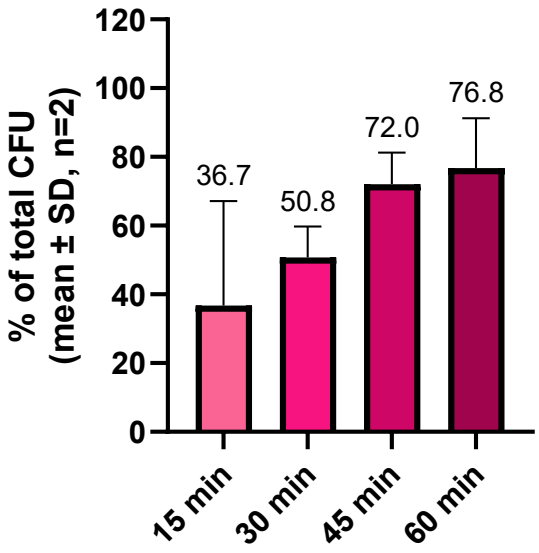



***Figure 9. Optimisation of incubation time for the capture of E. coli O157 by FluIDect Colligo Mag µBeads.*** *To determine the optimal incubation period, E. coli O157 was spiked at 100 CFU/mL into 300 µL of PBS and incubated with the antibody-functionalised magnetic µBeads at 2000 rpm in a thermomixer for 15, 30, 45 or 60 minutes; captured bacteria were subsequently quantified by plating. Capture efficiency increased progressively with incubation time, from 36.7% at 15 minutes to 50.8% at 30 minutes, 72.0% at 45 minutes and 76.8% at 60 minutes (mean ± SD, n = 2). Recovery at the shorter time points was not only lower but also markedly more variable, as reflected in the large standard deviation at 15 minutes, indicating that binding equilibrium had not yet been reached. The increase between 45 and 60 minutes was comparatively modest, suggesting that capture approaches saturation towards the end of the time course. Based on these data, an incubation time of 60 minutes was selected as the standard assay condition, providing the highest and most reproducible recovery of E. coli O157.*

## Conclusions

- ✓ **High capture efficiency:** *E. coli* O157 was captured at a rate of 76–83% across concentrations of $10^2$ to $10^4$ CFU/mL, with detection still achievable at 10 CFU/mL (73.4%).
- ✓ **Rapid workflow:** Target capture achieved after only 60 minutes of incubation, with no pre-enrichment required.
- ✓ **High target selectivity:** Low cross-reactivity (<10.1%) against *Salmonella enterica* serovar Typhimurium, a Gram-negative rod of comparable size, morphology and cell-envelope structure, corresponding to an approximately 8- to 12-fold preference for the intended target.
- ✓ **Matrix robustness:** Consistent performance in PBS, artificial urine, deionised water, turbid mud-spiked water and diluted milk (1%) (74.7–81.4%); reduced recovery in whey (51.7%) was partially restored by dilution (61.8% in 50% whey).
- ✓ **Storage stability:** Full activity retained after at least nine months of storage.
- ✓ **Flexible readout:** Compatible with plating, flow cytometry with nucleic acid staining, and downstream DNA extraction, allowing integration into existing laboratory workflows.

## Technical Summary

| Parameter | Value / Description |
|---|---|
| Target organism | *Escherichia coli* O157 |
| µBead material | Magnetic polystyrene |
| Detection method | Plating (CFU), flow cytometry (SYTO 85 nucleic acid stain), ELISA |
| Tested matrices | PBS, water, artificial urine, mud-spiked water, whey (100% and 50%) and 1% milk |
| Storage stability | ≥ 9 months, PBS + 0.02% $NaN_3$, 4 °C |
| Cross-reactivity | Low vs. *Salmonella enterica Typhimurium* (3.6–4.5-fold selectivity for *E. coli* O157) |
| Limit of detection | 10–100 CFU/mL |
| Capture time | 60 min |
| Sample input volume | 300 µL to 1 mL |
| Bead size | 3.1–7.2 µm |

## Ordering Information

FluIDect® Colligo Mag µBeads – anti-*E. coli* O157

Package sizes can be provided according to customer requests.

Find out more at https://fluidect.com/en/functionalised-beads


Call us +49-(0)3641 / 55 41 380
Email Us application@fluidect.com
Address Winzerlaer Str. 2;07745 Jena, Germany





Specifications subject to change without notice.
Copyright FluIDect GmbH.
For Research Use Only