MagIso™ E. coli Isolation Kit

Cat.No: WHK-V001
Lot. No. (See product label)

PRODUCT INFOMATION

Unit Size	20, 100 tests

DESCRIPTION

Description	Rapid detection (~2 hr) of fecal-indicator contamination in recreational waters is an important public health consideration in the monitoring and management of both freshwater and saltwater beaches. The MagIso™ E. coli Isolation Kit has been developed for the immunomagnetic separation and detection of E. coli from a wide range of sample matrices, including but not limited to wastewater and recreational waters. The high sensitivity and specificity of the MagIso™ E. coli Isolation Kit results from the combination of immunological identification and magnetic concentration.

APPLICATION

Application	Microbe Isolation
Application Notes	The MagIso™ E. coli Isolation Kit is intended for the immunomagnetic separation and estimation of E. coli from a wide range of sample matrices including wastewater and recreational waters.

Kit Components

Kit Contents	• anti-E. coli Antibody • Antibody Coated Magnetic Particles • Rinse Buffer • Somatic Cell Lysis Reagent • Bacterial Lysis Reagent • Luciferin-Luciferase Concentrate • Luciferin-Luciferase Diluent

Materials Required But Not Supplied

Materials Required But Not Supplied	• Tube Coating Solution (# WHK-V004) • Instrument Blank Solution (# WHK-V005) • Micropipettors and tips (100 uL, 1000 uL) • Serological pipettes (10 mL, 25 mL) • 50 mL Conical Centrifuge Tubes (sterile) • 1.5 mL Conical Microfuge Tubes with caps (sterile) • Magnets for collecting paramagnetic particles (50 mL size, 1.5 mL size) • Rotator (capable of rotating at 18 rpm). • Luminometer

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Specification

Sample Type	Including but not limited to wastewater and recreational waters.

Important Notes

Important Notes	For Research Use Only.

Procedure:

1. Prepare coated 50 ml conical tubes and 1.5 ml microfuge tubes. Determine the number of samples to be tested (and duplicates if desired) and coat one 50 ml conical tube and one 1.5 ml microfuge tube for each sample. Coat an addition 50 ml conical tube and 1.5 ml microfuge tube for the instrument blank.

NOTE: Because of processing times and magnet configurations, it may be useful to process samples in batches of six. If more than six samples need to be processed, the batches can be staggered every thirty minutes.

a. To coat the 50 ml conical tubes, transfer 10 ml of Tube Coating Solution to the first tube and swirl the liquid around the complete inner surface of the tube (including the lid).

b. After the first tube is coated, transfer the coating solution to the next 50 ml tube and repeat the process until all of the 50 ml tubes have been coated. Place tubes upright in tube rack as each is completed. Discard remaining coating solution.

c. Using a sterile 5 ml pipette, remove excess coating solution that may have collected in the bottom of the tubes.

d. To coat 1.5 ml microfuge tubes, transfer 700 µl of Tube Coating Solution to the first tube and then continue as with the 50 ml tubes until all the 1.5 ml microfuge tubes have been coated.

2. Determine the number of samples and instrument blanks to be tested. To insure adequate reagent, add one additional test to the total and prepare 1x Luciferin-Luciferase Reagent by diluting one part (50 µl) Luciferin-Luciferase Concentrate with 2 parts (100 µl) Luciferin-Luciferase Diluent for each sample and instrument blank. The calculated overage will assure adequate volume for each sample.

3. To begin the assay, transfer 25 ml of each sample into appropriately labeled and coated 50 ml conical tubes. Likewise, transfer 25 ml of the Instrument Blank Solution to the appropriate tube(s) by pouring directly from the bottle as supplied.

4. Add 100 µl of anti-E. coli Antibody to each tube, cap tightly, and rotate (~18 rpm) for 15 min.

5. After 15 min., remove the tubes from the rotator and add 200 µl of Antibody Coated Magnetic Particles to each tube.

6. Return the tubes to the rotator and mix for an additional 45 min.

7. After the 45 min. incubation with the Antibody Coated Magnetic Particles, remove the tubes from the rotator and transfer to a rack.

8. Place a tube in the 50 ml magnet holder and gently rock the tube by hand end-to-end through approximately 90°, tilting cap end and conical end of the tube up and down in turn. Continue the tilting action for 2 min. with approximately one tilt per second.

9. Lay the magnet on a flat surface, tube side up, for approximately 1 min.

10. Pick up the magnet and carefully loosen the lid without disturbing the beads.

11. Rotate so that the magnet is now on top and discard the supernatant by slowly and carefully pouring into a waste container. Do not disturb the beads on the magnet.

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12. Remove the tube from the magnet and add 700 ul of Rinse Buffer to the tube.

13. Using a 1 ml serological pipette, rinse the beads down the side of the tube, gently resuspend the beads, and transfer the bead/bacteria suspension into an appropriately labeled and coated 1.5 ml microfuge tube.

14. Rinse the 50 ml conical tube with an additional 300 µl of Rinse Buffer and transfer to the 1.5 ml microfuge tube.

15. Repeat steps 8-14 with the remaining samples and instrument blank(s).

16. Place six of the 1.5 ml microfuge tubes in the 1.5 ml tube magnet.

17. Gently rock the tubes through 180° by hand for approximately 2 min. at a rate of once per second.

18. When the solution is clear, discard the supernatant with a long-tipped Pasteur pipette. Remove as much liquid as possible, including the drop in the lid.

19. Remove the tubes from the magnet and add 200 µl of Somatic Cell Lysis Reagent to each sample.

20. Mix beads/bacteria with the SCLR by gently rotating the tubes with fingertips.

21. Place the tubes in the 1.5 ml tube magnet.

22. Repeat step 17 for approximately 1 min.

23. Repeat step 18.

24. Remove the tubes from the magnet and add 400 µl Rinse Buffer. Gently mix with fingertips. DO NOT VORTEX.

25. Repeat steps 21, 22, and 23.

26. Remove the tubes from the magnet and add 50 µl of Bacterial Lysis Reagent. Vortex each tube for 30 seconds and then allow to sit for 2 min.

27. Prepare luminometer for measurement.

28. After 2 min, place the tubes in the magnet and rotate as before for 1 min.

29. One sample at a time, while the tubes are still on the magnet, transfer all of the supernatant (including the drop in the lid) to a new, sterile, uncoated 1.5 ml microfuge tube.

30. Place the tube with supernatant into the luminometer, add 150 µl of 1x Luciferin-Luciferase Reagent (see Step 2), and gently mix 3 times with the pipettor. Immediately close lid and record Relative Luminescence Units.

31. Repeat steps 29 and 30 until all samples have been measured.

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