

***E. coli* O26 Immuno-fluorescence kit**

art. #: ID12307

size: kit for 100/200/400 assays

General information *E. coli* O26

With approximately 76 million outbreaks a year, food-related illnesses are a major public-health concern world-wide. More than 200 diseases are known to be transmitted by food, making food a key vector for disease transmission to humans. *Escherichia coli* O26 is an enterohemorrhagic (causing bloody diarrhea and colitis) strain of the bacterium *Escherichia coli* and a cause of foodborne illness. While most *E. coli* strains are harmless and normally found in the intestines of mammals, enterohemorrhagic *E. coli* strains may produce toxins and cause severe illness causing fatalities among susceptible groups like young children and elderly people. These enterohemorrhagic *E. coli* strains are collectively also known as EHEC bacteria. Transmission is via the fecal-oral route, and most illness has been associated with eating undercooked, contaminated ground beef, swimming in or drinking contaminated water, and eating contaminated vegetables. Infection often leads to hemorrhagic or nonhemorrhagic diarrhea, abdominal cramps, and occasionally to kidney failure, especially in young children and elderly persons.

Applications

Excellent suitable for immunofluorescence staining procedures on glass, other applications or platforms are not tested but should not be excluded.

Contents:

Content	Format	Use	Store at
Wash Buffer 1	Liquid	Ready to use	Room temperature
Blocking Buffer	Liquid	Ready to use	4°C, before use warm up to room temperature
Wash Buffer 2	Liquid	Ready to use	Room temperature

***E. coli* O26 antibody**

Clonality: : polyclonal
Immunogen : whole cells of *E. coli* serogroup O26
Host animal : goat
Conjugation : fluorescein analogue
Purification : affinity purified
Format : lyophilized

Stabilizer and preservative

IgG free bovine serum albumin (BSA) is added as a protein stabilizers. 0,02% sodium azide is added as a preservative. Non-sterile.

Antibody concentration

The concentration of affinity purified antibody is 0,5 mg as determined by UV absorbance at 280nm. Upon rehydration with water, the solution will contain 1% BSA, 100 mM phosphate, 150 mM sodium chloride, 0,02% sodium azide, pH 7,4. The conjugate will be at a concentration of 0,5mg/ml.

Rehydration

Rehydrate with 1 ml reagent quality water, rotate the vial until the lyophilized pellet is totally dissolved.

Use

Dilute to the desired concentration with blocking buffer immediately before use, mix thoroughly. This working solution is not recommended for long term storage.

Storage

Store at 2-8°C until rehydration, rehydrated antibody may be stored for up to one week at 2-8°C, thereafter it should be stored at -20°C. Avoid multiple freeze thaw steps. When aliquoting, store product in volumes greater than 50µl. Variations in temperature due to freeze cycles may cause loss of activity when rehydrated product is stored frozen in aliquots less than 50µl.

Specificity

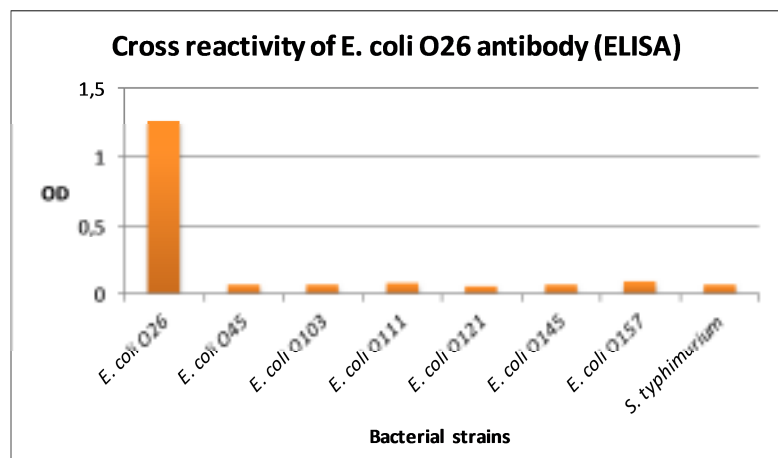
Tested by ELISA techniques as applicable (Figure). This product reacts highly specifically with *E. coli* O26, minimal cross-reactivity to other *E. coli* serogroups.

Excitation/emission values

Fluorescein analogue is excited at 493 nm (in PBS) and emits at 518 nm (in PBS).

Contact information

If you have any questions about this product, please contact us at Sales@innosieve.com or call us at (+31)-646717500.



Protocol in eppendorf tube:

Notes before starting:

- All centrifugation steps are performed for 2 minutes at 14.000 RCF (relative centrifugal force)
- Not all the supernatant is removed to prevent loss of the pellet

Method:

1. Pipette 500µl sample in an eppendorf tube
Remark: if the amount of sample is less, add Wash Buffer 1 to a final volume of 500µl
2. Vortex the sample and centrifuge
3. Remove 450µl of the supernatant without disturbing the pellet
4. Resuspend the pellet in the remaining supernatant
5. Add 500µl Blocking Buffer, vortex and centrifuge, remove 500µl of the supernatant
6. Resuspend the pellet in the remaining supernatant
7. Add 500µl Wash Buffer 1, vortex and centrifuge, remove 500µl of the supernatant
8. Resuspend the pellet in the remaining supernatant
9. Prepare the antibody solution, dilute for one sample 10µl antibody stock in 90µl Blocking Buffer, mix by pipetting up and down
10. Add 100µl prepared antibody working solution, vortex and incubate at room temperature for 10 minutes
11. Add 400µl Wash Buffer 1, vortex and centrifuge, remove 500µl of the supernatant
12. Resuspend the pellet in the remaining supernatant
13. Add 500µl Wash Buffer 1, vortex and centrifuge, remove 500µl of the supernatant
14. Resuspend the pellet in the remaining supernatant
Optional: in case higher stringency is required add 500µl Wash Buffer 2, vortex and centrifuge. Discard the supernatant and resuspend the pellet in the remaining supernatant. This step can be repeated. When applying this stringency step, finish by applying Wash Buffer 1 to resuspend the cells.
15. Add 5µl of the bacterial suspension onto a glass slide and allow to air dry in the dark
16. Heat fix the sample by passing the glass slide the flame for 3 or 4 times
17. Add Mounting Medium and cover glass
18. Analyze the sample

Protocol on glass slide

Notes before starting:

- Any type of sample can be used, preferably suspended cells in buffer

Method 1:

1. Add the sample onto a glass slide and allow to air dry in the dark
Note: in most cases a sample volume of 5 - 50 μ l is recommended
2. Heat fix the sample by passing the glass slide the flame for 3 or 4 times
3. Add 50 μ l blocking buffer, allow to stand 1 minute, rinse gently with 500 μ l wash buffer 1
4. Prepare the antibody solution, dilute for one sample 5 μ l antibody stock in 45 μ l blocking buffer, mix by pipetting up and down
5. Add 50 μ l diluted antibody, pipette gently up and down 8 times and incubate at RT for 10 minutes
Note: depending on the sample the incubation time can be elongated to improve the signal
6. Rinse twice gently with 500 μ l wash buffer 1
Optional: in case higher stringency is required rinse gently with 500 μ l Wash Buffer 2. This step can be repeated. When applying this stringency step, finish with one wash step using Wash Buffer 1
7. Allow to air dry the sample in the dark
8. Add Mounting Medium and cover glass
9. Analyze the sample

Protocol on glass slide

Notes before starting:

- Any type of sample can be used, preferably suspended cells in buffer

Method 2:

1. Add the sample onto a glass slide and allow to air dry in the dark
Note: in most cases a sample volume of 5 - 50 μ l is recommended
2. Heat fix the sample by passing the glass slide the flame for 3 or 4 times
3. Rinse twice gently with 500 μ l MilliQ
4. Add 50 μ l Wash Buffer 3, allow to stand 2 minutes, rinse gently with 500 μ l Wash Buffer 3
5. Rinse gently with 500 μ l blocking buffer
6. Prepare the antibody solution, dilute for one sample 5 μ l antibody stock in 45 μ l Blocking Buffer, mix by pipetting up and down
7. Add 50 μ l diluted antibody, pipette gently up and down 8 times and incubate at RT for 10 minutes
8. *Note: depending on the sample the incubation time can be elongated to improve the signal*
9. Rinse thrice gently with 500 μ l Wash Buffer 1
Optional: in case higher stringency is required rinse gently with 500 μ l Wash Buffer 2. This step can be repeated. When applying this stringency step, finish with one wash step using Wash Buffer 1
10. Allow to air dry the sample in the dark
11. Add Mounting Medium and cover glass
12. Analyze the sample