

***Campylobacter spp* Immuno-fluorescence kit**

art. #: ID12305

size: kit for 100/200/400 assays

General information *Campylobacter spp*

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. *Campylobacter*, the causative agent of Campylobacteriosis, is currently the most commonly cause of foodborne disease outbreaks and one of the most common causes of diarrheal illness in the United States. The vast majority of cases occur as isolated, sporadic events and not as part of recognized outbreaks. Many cases therefore, go undiagnosed or unreported, but Campylobacteriosis is estimated to affect over 2.4 million persons every year in the United States. Although *Campylobacter* does not commonly cause death, it has been estimated that approximately 124 persons with *Campylobacter* infections die each year. *Campylobacter* organisms are spiral-shaped bacteria that can cause disease in humans and animals. Most human illness is caused by one species, called *Campylobacter jejuni*, but human illness can also be caused by other species. Most cases of Campylobacteriosis are associated with eating raw or undercooked poultry meat or from cross-contamination of other foods by these items. Outbreaks of *Campylobacter* are usually associated with unpasteurized milk or contaminated water. Humans infected with *Campylobacter* typically suffer from diarrhea, cramping, abdominal pain, and fever within two to five days after exposure to the organism. Some infected persons do not have any symptoms. In persons with compromised immune systems, *Campylobacter* occasionally spreads to the bloodstream and causes a serious life-threatening infection.

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

***Campylobacter spp* antibody**

Clonality: : polyclonal
Immunogen : different strains of heat-killed whole cells of *Campylobacter*
Host animal : goat
Conjugation : Fluorescein
Purification : affinity purified
Format : lyophilized

Stabilizer and preservative

Goat serum and bovine serum albumin (BSA) are added as a protein stabilizers. No preservatives added. Additional biological protection may be provided with 0,1% sodium azide. Non-sterile.

Antibody concentration

This product contains 0,5 mg of affinity purified antibody.

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

This antibody is highly specific and is broadly reactive to *Campylobacter* spp. and recognizes the *Campylobacter* strains *C. coli*, *C. concisus*, *C. fetus*, *C. hyointestinalis*, *C. jejuni*, *C. laridis* and *C. mucosalis*. The intensity of the reaction may vary with the strain of *Campylobacter* tested. It may also have some cross-reactivity to related bacteria (i.e. *Helicobacter*) when tested by direct fluorescent antibody slide assay.

Excitation/emission values

Fluorescein is excited at 494 nm (in PBS) and emits at 521 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