

Salmonella spp. Immuno-fluorescence kit

art. #: ID12318

size: kit for 100/200/400 assays

General information *Salmonella 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. *Salmonella*, the causative agent of Salmonellosis, is currently the second most commonly cause of foodborne disease outbreaks after *Campylobacter*. In 2010, *Salmonella* alone was responsible for 1,4 million disease outbreaks a year, resulting in approximately 158.000 visits to physicians, 15.000 hospitalizations, 415 deaths and an estimated economical loss of 2,7 billion Dollars in the United States. Almost all known human pathogenic *Salmonella* bacteria are designated to the subspecies *enterica*, which comprises over 2.500 serovars or serotypes. *Salmonella* bacteria generally infects humans through the consumption of contaminated food of animal origin, although fresh vegetables are increasingly implicated in *Salmonella* outbreaks since contamination may occur already in the field when manure is used for soil fertilization. Humans infected with *Salmonella* typically suffer from abdominal pain, diarrhea and fever while some people develop potentially lethal bloodstream infections, leading to sepsis.

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

Salmonella spp. antibody

Clonality : polyclonal
Immunogen : common structural antigen (CSA-1)
Host animal : goat
Conjugation : fluorescein
Purification : affinity purified
Format : lyophilized

Stabilizer and preservative

Bovine serum albumin (BSA) is added as a protein stabilizer. No preservatives added. Additional biological protection may be provided with 0,01 - 0,05% sodium azide. Non-sterile.

Antibody concentration

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

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