Legionella pneumophila type 1 Immuno-fluorescence kit

art. #: ID12315
size: kit for 100/200/400 assays

General information *Legionella spp.*
Legionella spp are Gram negative bacteria which are the causative agent of Legionnaires' disease (most notably *L. Pneumophila* type 1). *Legionella* spp are common waterborne organisms, and devices such as cooling towers, hot water systems and spas have been associated with outbreaks of infection. It is estimated that in the United States alone there are between 10,000 and 50,000 cases of Legionnaires' disease each year (U.S. Department of Labor). Once inside a host, incubation may take up to several weeks. Initial symptoms are flu-like, including fever, chills, and dry cough. During the more advanced stages the disease causes problems with the gastrointestinal tract, the nervous system and advanced symptoms of pneumonia may also present. The case–fatality rate of the Legionnaires' disease is variable depending on susceptibility of the patient, but is reported to be as high as 40-80% in hospitalized patients (source, world health organization). Consequently, it should be actively checked for in the water systems of hospitals and nursing homes.

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

Legionella pneumophila type1 antibody

Clonality: : polyclonal
Immunogen : various strains of heat-killed *Legionella*
Host animal : rabbit
Conjugation : fluorescein analogue
Purification : protein G purified
Format : lyophilized

Stabilizer and preservative
IgG free bovine serum albumin (BSA) is added as a protein stabilizer, 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, 100mM phosphate, 150mM sodium chloride, 0,02% sodium azide, pH 7,4. The conjugate will be at a concentration of 0,5 mg/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
This antibody broadly reacts to *Legionella pneumophila* type 1, while demonstrating minimal cross-reactivity to related bacteria (*Citrobacter, Salmonella, Escherichia* and *Yersinia*).

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 stringently 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 µl is recommended

2. Heat fix the sample by passing the glass slide the flame for 3 or 4 times

3. Add 50µl blocking buffer, allow to stand 1 minute, rinse gently with 500µl wash buffer 1

4. Prepare the antibody solution, dilute for one sample 5µl antibody stock in 45µl blocking buffer, mix by pipetting up and down

5. Add 50µl 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 500µl wash buffer 1
   Optional: in case higher stringently is required rinse gently with 500µl 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 µl is recommended
2. Heat fix the sample by passing the glass slide the flame for 3 or 4 times
3. Rinse twice gently with 500µl MilliQ
4. Add 50µl Wash Buffer 3, allow to stand 2 minutes, rinse gently with 500µl Wash Buffer 3
5. Rinse gently with 500µl blocking buffer
6. Prepare the antibody solution, dilute for one sample 5µl antibody stock in 45µl Blocking Buffer, mix by pipetting up and down
7. Add 50µl 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 500µl Wash Buffer 1
   Optional: in case higher stringently is required rinse gently with 500µl 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