

Read this Instructions For Use carefully before testing.

For in vitro diagnostic use only

MIZUHO MEDY Co., Ltd.

*Streptococcus pneumoniae* antigen kit • *Legionella pneumophila* antigen kit

## Quick Chaser *Streptococcus pneumoniae*/Legionella

### [General precautions]

- 1) For in vitro diagnostic use only.
- 2) The diagnosis of *Streptococcus pneumoniae* infection and legionellosis should be comprehensively made not only by test result of this product, but also in conjunction with the assessment of clinical progress and results of other tests.
- 3) Procedures which are not described in instruction for use, are not guaranteed.
- 4) Cerebrospinal fluid can be used as a specimen for a detection of *Streptococcus pneumoniae* only. Do not use cerebrospinal fluid for a detection of *Legionella pneumophila*, since it is not included in Intended Use.

### [Package]

68250 : Quick Chaser Streptococcus pneumoniae/Legionella  
- 10 tests/kit

### [Contents]

- 1) Test plate - 10 tests
  - Rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies
  - Rabbit monoclonal anti-*Streptococcus pneumoniae* antibodies
  - Rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS antibodies
  - Colloidal gold conjugated to rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies
  - Colloidal gold conjugated to rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS antibodies
- 2) Dropper - 10 pieces

### [Intended Use]

For detection of *Streptococcus pneumoniae* antigen in urine or cerebrospinal fluid

(An aid of diagnosis for *Streptococcus pneumoniae* infection)

For detection of *Legionella pneumophila* serogroup 1 LPS antigen in urine

(An aid of diagnosis for legionellosis)

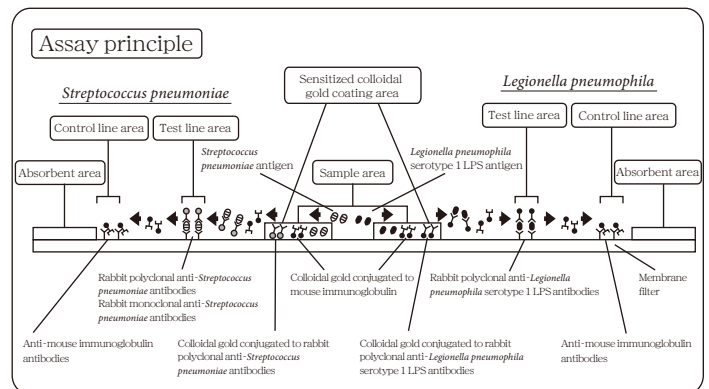
### [Principle of the test]

“Quick Chaser *Streptococcus Pneumoniae*/Legionella” is the in vitro reagent for detection of *Streptococcus pneumoniae* antigen and *Legionella pneumophila* serogroup 1 LPS based on Immunochromatographic Assay.

Colloidal gold conjugated to rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies or colloidal gold conjugated to rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS and colloidal gold conjugated to mouse immunoglobulin for control line are coated in sensitized colloidal gold coating area in test plate. And rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies and rabbit monoclonal anti-*Streptococcus pneumoniae* antibodies in *Streptococcus pneumoniae* test line area and rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS antibodies in *Legionella pneumophila* test line are immobilized. Anti-mouse immunoglobulin antibodies for each control line are immobilized in control line area.

According to immunochromatographic principle, when samples are added to the sample area, in the presence of *Streptococcus pneumoniae* antigens or *Legionella pneumophila* serogroup 1 LPS antigen, they migrate to the area between sample area and test line area, where reacting with colloidal gold conjugated to rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies or colloidal gold conjugated to rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS antibodies and moreover, react with rabbit polyclonal anti-*Streptococcus pneumoniae* antibodies and rabbit monoclonal anti-*Streptococcus pneumoniae* antibodies or rabbit polyclonal anti-*Legionella pneumophila* serogroup 1 LPS antibodies and they are caught in each test line area. As a result, purple-red line appears in each test line area.

Purple-red line also simultaneously appears for catching colloidal gold conjugated to mouse immunoglobulin by anti-mouse immunoglobulin antibodies in each control line area, regardless of presence of *Streptococcus pneumoniae* or *Legionella pneumophila* serogroup 1 LPS.



### [Procedural precautions]

- 1) Use urine or cerebrospinal fluid as a specimen and do not use , serum, sputum or pharyngeal swab etc.
- 2) Do not use turbid specimen containing pus or blood etc.
- 3) Collect specimen with an aseptic container.
- 4) Collected specimen should be tested as soon as possible. In case that test is not performed immediately or specimen need to be stored for a long time, test specimen within 3 days for storage at 5 ~ 30 °C and within 14 days for storage at - 80 ~ 4 °C. Do not repeat freezing and thawing of specimen three times or more. Specimen should be brought to 15 ~ 30 °C prior to use.
- 5) Dropper included in kit is not sterilized. When performing culture test, please separate specimens in advance to avoid contamination of specimens.
- 6) Add fixed volume (about 130 μL) of specimen to the center of sample area. As for the case except fixed volume, an accurate reaction may not be conducted.
- 7) Bring test plate to 15 ~ 30 °C prior to testing.
- 8) Please be sure to observe the interpretation time as it will cause false negatives and false positives.
- 9) Interfering substances and medications

The following substances and blood did not interfere the performance of this product at the concentration listed below:

Glucose (5000 mg/dL)  
Albumin (2000 mg/dL)  
Ascorbic acid (500mg/dL)  
Acetylsalicylic acid (2000 mg/dL)  
Ibuprofen (500mg/dL)  
Sodium chloride (3000 mg/dL)  
Calcium chloride (110mg/dL)  
Urea (5000mg/dL)  
Cold medicine (acetaminophen concentration) (500mg/dL)  
Blood (2%)  
Bilirubin(10mg/dL)

10) Cross reactivity

Cross reactivity with the following bacteria and virus were not observed.

• Bacteria

*Candida albicans*, *Chlamydomphila (Chlamydia) pneumoniae*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Haemophilus influenzae a*, *Haemophilus influenzae b*, *Haemophilus influenzae c*, *Haemophilus influenzae d*, *Haemophilus influenzae e*, *Haemophilus influenzae f*, *Klebsiella pneumoniae*, *Legionella feeleii*, *Legionella longbeachae*, *Legionella rubrilucens*, *Legionella londiniensis*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis A*, *Neisseria meningitidis B*, *Neisseria meningitidis C*, *Neisseria meningitidis Y*, *Neisseria meningitidis W-135*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus anginosus*, *Streptococcus Group A*, *Streptococcus Group B*, *Streptococcus Group C*, *Streptococcus Group F*, *Streptococcus Group G*, *Streptococcus mutans*

• Virus

Adenovirus 3, Adenovirus 11, Adenovirus 19, Adenovirus 37, Coronavirus, Coxsackievirus A7, Coxsackievirus B3, Echovirus, Epstein-Barr virus, Herpes simplex virus 2, Influenzavirus A, Influenzavirus B, Measles virus, Mumps virus, Parainfluenza virus 1, Respiratory syncytial virus A, Respiratory syncytial virus B, Rhinovirus 8, Rotavirus, Rubella virus, Varicella zoster virus

In addition, cross reaction with *Streptococcus mitis* was observed at  $1 \times 10^7$  CFU/mL or more.

11) Reactivity with *Legionella pneumophila* serotype

The reactivity with *Legionella pneumophila* serotype is shown in the following table:

Specimen	Specimen concentration (CFU/mL)			
	Quick Chaser Streptococcus pneumoniae/Legionella			
	$1 \times 10^9$	$1 \times 10^8$	$1 \times 10^7$	$1 \times 10^4$
<i>Legionella pneumophila</i> SG1	Positive	Positive	Positive	Positive
<i>Legionella pneumophila</i> SG2	Positive	Positive	Negative	Negative
<i>Legionella pneumophila</i> SG3	Positive	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG4	Positive	Positive	Negative	Negative
<i>Legionella pneumophila</i> SG5	Positive	Positive	Negative	Negative
<i>Legionella pneumophila</i> SG6	Positive	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG7	Positive	Positive	Positive	Negative
<i>Legionella pneumophila</i> SG8	Negative	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG9	Positive	Positive	Negative	Negative
<i>Legionella pneumophila</i> SG10	Positive	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG11	Negative	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG12	Negative	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG13	Negative	Negative	Negative	Negative
<i>Legionella pneumophila</i> SG15	Positive	Positive	Negative	Negative

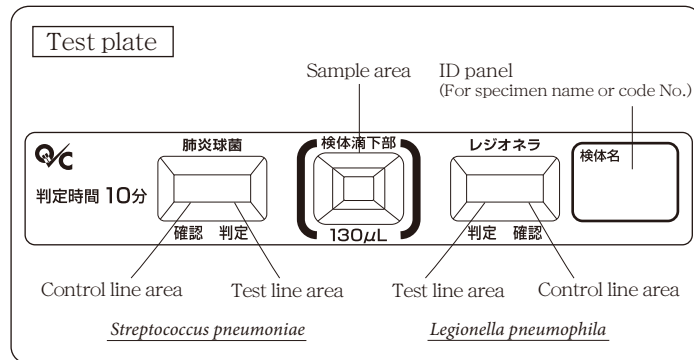
From the table, cross reactivity was observed in *Legionella pneumophila* serotypes 2, 3, 4, 5, 6, 7, 9, 10, 15. In the literature of Barthe et al.<sup>1)</sup> and Dagmar et al.<sup>2)</sup>, LPS antigen of *Legionella pneumophila* has been shown to have a common antigen regardless of serotype.

12) Influence of vaccine

When *Streptococcus pneumoniae* vaccine is inoculated, antigens derived from vaccine are exhausted in urine for a few days and be careful because positive test result may be given.<sup>3)</sup>

[Test procedure]

●Details of test plate



●Test procedure

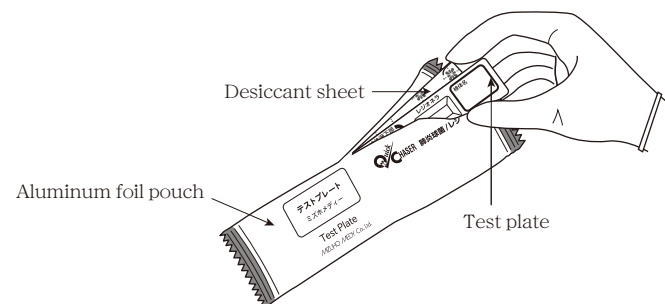
1) Preparation of reagent

No prior preparation required for test plate.

2) Test procedure

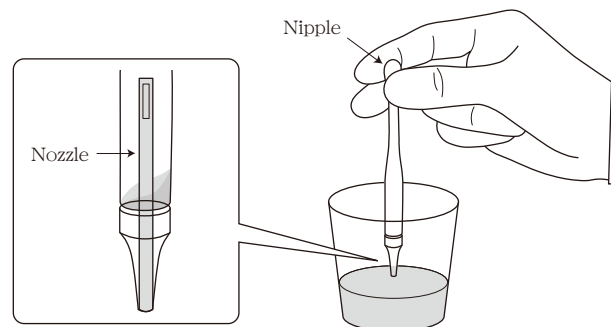
① Remove test plate from aluminum foil pouch.

Discard desiccant sheet included in aluminum foil pouch.

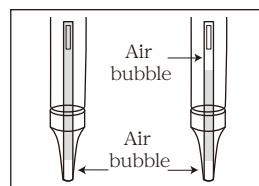


② Pick up the nipple part of the dropper included in the kit and suck up a sufficient amount of specimen so that the nozzle can be filled with one suction while paying attention for the tip of the dropper not to get out of the specimen.

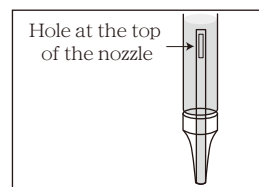
The specimen sucked up excessively overflows inside the dropper from the nozzle part and remains in the dropper.



Note) in the case as shown below, correct amount of specimen cannot be dropped.

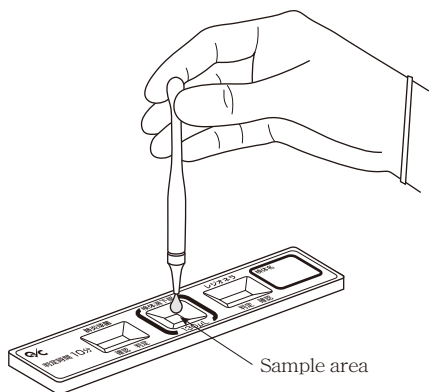


If air bubbles enter the nozzle, discharge and fill the nozzle again.



Do not suck up the specimen repeatedly until the specimen overflowing from the nozzle part exceeds the hole at the top of the nozzle part. If the specimen exceeds the hole at the top of nozzle part, collect the specimen with a new dropper.

- ③ Drop the specimen by pushing the nipple part of the dropper to the sample area of the test plate. The required amount (about 130  $\mu$ L) of specimen is discharged.



※ If using a micropipette etc., drop 130 $\mu$ L of specimen onto the test plate.

- ④ Leave to react at 15 ~ 30  $^{\circ}$ C.  
Visually interpret test result by lines appearing in test line area and in control line area after 5 ~ 10 minutes.

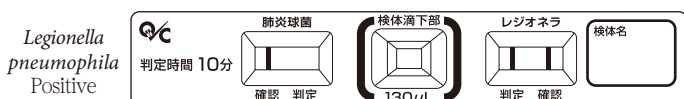
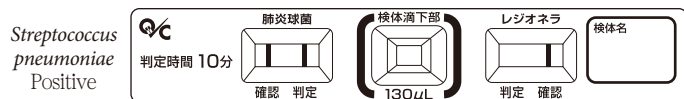


## [Interpretation]

Interpret by existence of red-purple lines appearing in test line area and control line area.

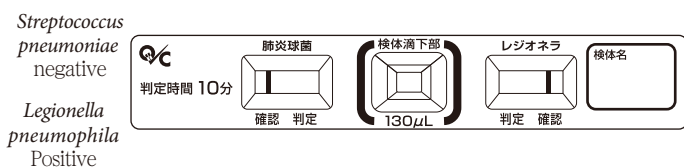
《Positive》

Both each test line and control line appear.



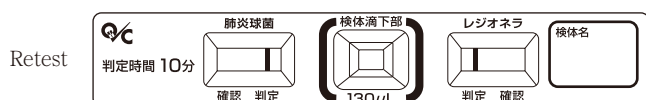
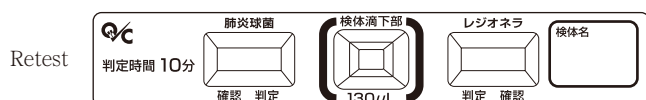
《Negative》

Only control line appears.



《Retest》

If no control line appears on each side, an operational failure can be considered, e.g. an insufficient sample volume. Recheck test procedure and retest with new test plate. If the same result comes out in the retest again, please try another method.



## ● Interpretational precautions

- In case both test line for *Streptococcus pneumoniae* or *Legionella pneumophila* and control line appear at 5 ~ 10 minutes after adding sample, it can be interpreted as *Streptococcus pneumoniae* positive or *Legionella pneumophila* positive. Negative should be interpreted at 10 minutes after adding sample. Streak line may appear temporarily due to flow of colloidal gold, but please note that this is not a test line. After interpretation time, colloidal gold may appear line-wise due to drying of test plate over time, so interpret test results at the predetermined time.
- This product is used as an aid in the diagnosis for infection of *Streptococcus pneumoniae* and legionellosis. In case that *Streptococcus pneumoniae* antigens or *Legionella pneumophila* serotype 1 LPS antigens in specimen are below the detection limit of the test, test result could be interpreted as negative, even though patients are infected by *Streptococcus pneumoniae* or *Legionella pneumophila*. Moreover, special factors in specimen could cause non-specific reaction and negative specimen could be interpreted as positive. The definitive diagnosis should be made comprehensively in conjunction with the assessment of clinical progress and other test result.
- In case test lines appear in both *Streptococcus pneumoniae* area and *Legionella pneumophila* area, there is the possibility of overlap infection of *Streptococcus pneumoniae* and *Legionella pneumophila*, but please recheck for confirmation. In addition, the diagnosis should be made comprehensively in conjunction with the assessment of clinical progress and other test result.
- Streptococcus mitis* has a common antigen with *Streptococcus pneumoniae* and it is supposed that positive result is given when there is much quantity of the antigens.
- When *Streptococcus pneumoniae* habitually resides to the nasopharynx in infant, *Streptococcus pneumoniae* antigens are excreted in urine and be careful because positive result may be given.
- It is usually said that the amount of *Streptococcus pneumoniae* antigen and *Legionella pneumophila* antigen in the urine will reach detectable amount or higher after 3 days from the appearance of pneumoniae symptoms, but it depends on the case. In addition, antigens may be excreted in urine from a few days to several weeks after infection. When interpreting the measurement results, consider the past medical history and clinical symptoms carefully and do not use it to determine the treatment effect.
- This product uses rabbit antibody as a raw material. Please note that patients receiving treatment with rabbit antiserum may cause false positives.

## [Performance characteristics]

### 1) Performance

#### ① Sensitivity

- When in-house *Streptococcus pneumoniae* positive control <sup>Note 1)</sup> was tested, a positive result was shown.
- When in-house *Legionella pneumophila* positive control <sup>Note 2)</sup> was used, a positive result was shown.

#### ② Accuracy

- When in-house *Streptococcus pneumoniae* positive control was tested, a positive result was shown.
- When in-house *Legionella pneumophila* positive control was used, a positive result was shown.
- When in-house negative control <sup>Note 3)</sup> was tested, a negative result was shown.

#### ③ Reproducibility

- When in-house *Streptococcus pneumoniae* positive controls were tested three time simultaneously, positive results were shown in all cases.
- When in-house *Legionella pneumophila* positive control were used three time simultaneously, positive results were shown in all cases.

- When in-house negative controls were tested three times simultaneously, negative results were shown in all cases.
- Note 1) *Streptococcus pneumoniae* antigen control solution, diluted by in-house negative control to be equivalent to  $6.25 \times 10^1$  CFU/mL of reference material
- Note 2) *Legionella pneumophila* antigen control solution, diluted by in-house negative control to be equivalent to  $2 \times 10^4$  CFU/mL of reference material
- Note 3) An aqueous solution containing urea, sodium chloride or the like

④ Detection limit

*Streptococcus pneumoniae*

$1.56 \times 10^1$  CFU/mL (ATCC49619)

*Legionella pneumophila*

$5.0 \times 10^3$  CFU/mL (ATCC33152)

⑤ *Streptococcus pneumoniae* serotype and reactivity

It is confirmed that this product reacts with *Streptococcus pneumoniae* serotype 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7F, 7C, 8, 9N, 9V, 10A, 11A/E, 12F, 13, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19F, 19A, 20, 22F, 23F, 23A, 24F, 24B, 28F, 29, 31, 33F, 34, 35B, 37, 38.

2) Correlations

● Urine

<*Streptococcus pneumoniae*>

Comparison with existing approval product (immunochromatographic assay)

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (1)	Positive	51	0	51
	Negative	8 <sup>※1</sup>	118	126
	Total	59	118	177

Sensitivity : 100%(51/51)  
Specificity : 93.7%(118/126)  
Accuracy : 95.5%(169/177)

※1 Five out of eight mismatched cases were also positive by other product (2).

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (2)	Positive	56	1 <sup>※3</sup>	57
	Negative	3 <sup>※2</sup>	117	120
	Total	59	118	177

Sensitivity : 98.2%(56/57)  
Specificity : 97.5%(117/120)  
Accuracy : 97.7%(173/177)

※2 Three mismatched cases were negative by other product (1).  
※3 One mismatched case was negative by other product (1).

<*Legionella pneumophila*>

Comparison with existing approval product (immunochromatographic assay)

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (3)	Positive	53	0	53
	Negative	0	124	124
	Total	53	124	177

Sensitivity : 100%(53/53)  
Specificity : 100%(124/124)  
Accuracy : 100%(177/177)

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (4)	Positive	51	0	51
	Negative	2 <sup>※2</sup>	124	126
	Total	53	124	177

Sensitivity : 100%(51/51)  
Specificity : 98.4%(124/126)  
Accuracy : 98.9%(175/177)

※4 Two mismatched cases were positive by other product (3).

● Cerebrospinal fluid

<*Streptococcus pneumoniae*>

Correlation with existing approval product (Immunochromatographic assay)

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (1)	Positive	27	0	27
	Negative	0	54	54
	Total	27	54	81

Sensitivity : 100%(27/27)  
Specificity : 100%(54/54)  
Accuracy : 100%(81/81)

Correlation with existing approval product (Latex agglutination method)

		Quick Chaser Streptococcus Pneumoniae/Legionella		
		Positive	Negative	Total
Other product (2)	Positive	15	0	15
	Negative	12 <sup>※5</sup>	54	66
	Total	27	54	81

Sensitivity : 100%(15/15)  
Specificity : 81.8%(54/66)  
Accuracy : 85.2%(69/81)

※5 Twelve unmatched cases were determined as positive by Other product (1).

3) Calibration reference material (Standard material)

*Streptococcus pneumoniae* (ATCC49619)

*Legionella pneumophila* (ATCC33152)

[Precautions for use or handling]

1) Precautions for handling (hazard prevention)

- Other infectious materials could be included besides *Streptococcus pneumoniae* and *Legionella pneumophila* in specimen. Be careful of handling specimen as potentially infectious materials.
- Be careful not to touch specimen directly to skin or not to get into eyes in wearing glasses, disposable gloves or mask at the time of use.
- If specimen is got into eyes or mouth, flush with a plenty of water as emergency treatment and see a doctor if necessary.
- Raw material of membrane which is used for test plate, is nitrocellulose. Do not perform test near fire because nitrocellulose is extremely flammable material.
- Wipe off with ethanol for disinfectant in case of getting splattered with sample (specimen).

2) Precautions for use

- Avoid freezing of reagent and store it in accordance with description of instruction for use. Do not use frozen reagents because they could show false results by change of quality.
- Do not use this product beyond expiration date.
- Use the test plate immediately after opening aluminum foil pouch. If test plate is left for a long time, it could not react by exposure to moisture.
- Do not touch sample area, test line area and control line area by hands directly.
- Do not perform test in the place such as under air conditioner where the dry wind directly blows the surface of the test plate, to prevent uneven migration.
- Do not use the reagent and the accessories etc. of this product except the purpose of this testing.
- Test plate and dropper are intended for single use only.

3) Precautions for waste disposal

- Treat liquid waste and used utensils by any one of following methods because specimen could contain other infectious material besides *Streptococcus pneumoniae*.
  - Immerse in sodium hypochlorite solution (effective chlorine concentration of 1,000 ppm) for 1 hour or more
  - Immerse in 2 % glutaraldehyde solution for 1 hour or more
  - Autoclave at 121 °C for 20 minutes or more
- Regarding disposal of used reagent and utensils, dispose of them as medical waste, industrial waste or infectious waste in accordance with Local Regulation and Law of waste disposal.

[Storage and Expiry]

- Storage : 1~30°C
- Expiry : 24 months (As indicated on package)

[References]

- Barthe C. et al. : Common epitope on the lipopolysaccharide of *Legionella pneumophila* recognized by a monoclonal antibody, J. Clin. Microbiol. 26(5) : 1016-1023,1988
- Dagmar J et al. : Cross-reacting lipopolysaccharide antigens in *Legionella pneumophila* serogroups 1 to 14, Infection and Immunity 63(6) : 2180-2184, 1995
- Kohler RB et al., J Clin Microbiol,20(4),605~607(1984)

Technical information  
Telephone +81-942-85-3845

Manufacturer: Mizuho Medy Co.,Ltd.

5-4 Fujinoki-machi, Tosu City, Saga, 841-0048 Japan

<https://www.mizuho-m.co.jp/en>