













Serazym[®] Anti-Francisella tularensis

Enzyme immunoassay for the qualitative detection of IgG, IgM, and IgA antibodies against lipopolysaccharide (LPS) of *Francisella tularensis* in serum of human origin

REF	E-049		96
IVD	In-vitro-diagnostic medical device	CE	

 **Seramun Diagnostica GmbH** • Spreehagener Str. 1 • 15754 Heidesee • Germany
 T +49 33767 791-10 • info@seramun.com • www.seramun.com

 Manufacturer	 Date of manufacture	 Use by date
LOT Batch number	REF Article number	SN Serial number
 Keep away from sunlight	 Temperature limits	 Biological risks
 Do not reuse	 Consult instructions for use	 Caution
IVD <i>In vitro</i> diagnostic medical device	 Sufficient for <n> tests	UDI Unique device identifier

Intended Use

Serazym® Anti-*Francisella tularensis* is an IVD test for the qualitative determination of antibodies of the IgG, IgM and IgA isotypes against lipopolysaccharide (LPS) of *Francisella tularensis* in serum of human origin through manual and semi-automatic processing by a laboratory professional user.

It is intended to aid in the diagnosis of tularemia in specimen materials from patients with suspicion of tularemia.

The test must not be used with specimen materials other than serum of human origin, for monitoring, screening, prediction, prognosis, as companion diagnostic, in the near patient setting and by lay persons.

Principle of the Test

Serazym® Anti-*Francisella tularensis* is an enzyme immunoassay in which the diluted serum samples, the two positive and the negative controls react with the lipopolysaccharide of *Francisella tularensis* adsorbed to the solid phase. After a 30-minute incubation at 37 °C unbound components are removed by a wash step. In the second incubation step horseradish peroxidase (HRP)-labeled anti-human IgG/IgM/IgA antibodies bind to the sample antibodies. After a 15-minute incubation at 37 °C unbound conjugate is removed by a wash step. HRP converts the colorless substrate solution to a blue reaction product in the following 10-minute enzymatic reaction step. The reaction is stopped by addition of the stop solution, resulting in a color change from blue to yellow. The optical density (OD) of the reaction product measured at 450 nm measuring and ≥ 620 nm reference filter is directly proportional to the concentration of specifically bound antibodies.

Test Components (Delivery Scope)

			For 96 wells
1	WELLS	Microtiter plate coated with LPS of < 10.0 mg/mL <i>Francisella tularensis</i>	12 single breakable 8-well strips, colorless, vacuum-sealed with desiccant
2	WASHBUF (10x)	Wash buffer (10x) Seramun® Wash buffer K TRIS based buffer	100 mL concentrate for 1000 mL solution, colorless, white cap
3	DILUENT	Sample diluent Seramun® Sample diluent P TRIS based buffer	100 mL, ready to use, colored orange/red, black cap
4	CONTROL +	Positive control Diluted human serum (inactivated); Absorbance see Certificate of Analysis	0.5 mL, ready to use, colored blue, red cap
5	CONTROL -	Negative control Diluted human serum (inactivated); Absorbance see Certificate of Analysis	0.5 mL, ready to use, colored blue, green cap
6	CONJ HRP	HRP conjugate < 0.1 µg/mL HRP labeled anti-human IgG-F(ab)2 (goat), < 0.1 µg/mL anti-human IgM (sheep), < 0.1 µg/mL anti-human IgA antibodies (sheep)	15 mL, ready to use, colored green, red cap
7	SUBSTR	Substrate SeramunBlau® fast2 < 0.1 % 3,3',5,5'-tetramethylbenzidine < 0.05 % hydrogen peroxide	15 mL, ready to use, colorless, blue cap

8	STOP	Stop solution SeramunBlau® stop 0.25 M sulphuric acid	15 mL, ready to use, colorless, yellow cap
9	COVER	Covering film	2 pieces
10		Certificate of Analysis	1 piece
11		Instructions for Use	1 piece

Additional Materials and Aids Required for the Test Procedure

Adjustable single-channel micropipette • 8-channel micropipette or multi-channel micropipette with pipette tips • reagent container for multi-channel micropipettes • 8-channel wash comb with vacuum pump and waste bottle or microplate washer • microplate reader with 450 nm measuring filter and ≥ 620 nm reference filter • deionized water • measuring cylinder • tubes for sample preparation

Important Information



This device is for *in-vitro* diagnostic use only. Follow the instructions carefully. The kit may be performed by laboratory professionals only.

Do not use reagents from damaged packages or bottles. Imprinted expiry dates must be observed. Do not mix components with reagents from other manufacturers.

Mixing of test kit components of different lots is only allowed for wash buffer (10x), substrate and stop solution.

All serious incidents occurring in relation with Serazym® Anti-Francisella tularensis must be reported to the manufacturer and the competent authority of the EU member state in which user and/or patient are located.

Information on Assay Procedure

All reagents should be stored at 2...8 °C. After incorrect storage test should no longer be used. Bring all test components to room temperature before use. Reagents that appear to be contaminated should not be used.

Each well of a microtiter plate can only be used once. Each sample and control has to be pipetted with a new pipette tip. Positive and negative controls are ready to use.

For larger sample series, pipetting reagents from liquid reservoirs using a multi-channel micropipette is recommended to avoid time delays and contaminations. Follow the pipetting scheme and time schedules of the protocol.

The aspiration and washing steps can be performed manually or with the help of a microplate washer or waterjet pump. Wash solution should be allowed a minimum reaction time of 5 s in the wells per wash cycle. Remove wash buffer residues by thoroughly aspirating or tapping dry the cavities.

Protect substrate from light! The test should not be performed with a substrate solution that is colored dark or contains colored precipitates.

Safety Instructions

Do not swallow reagents and avoid contact with mucous membranes.

Handle all components, patient samples and positive control as if potentially hazardous and infectious. Used wash buffer, other test kit components, and all materials that have come into contact with samples and reagents should be collected in appropriate containers and disposed of according to local and national regulations.

Information on hazardous ingredients and further details on the individual components of the test kit can be found in the following table.

Test component	Hazard labelling and supplementary information on ingredients
WELLS	Contains material of animal origin.
WASHBUF (10x)	EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction. EUH210: Safety data sheet available on request. Preservatives: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
DILUENT	EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction. EUH210: Safety data sheet available on request. Contains material of animal origin. Preservatives: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
CONTROL +	EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction. EUH210: Safety data sheet available on request. Contains material of animal origin. Preservatives: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane
CONTROL -	EUH208: Contains reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1). May produce an allergic reaction. EUH210: Safety data sheet available on request. Contains material of animal origin. Preservatives: < 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane
CONJ HRP	Preservatives: < 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); < 0.1 % 5-bromo-5-nitro-1,3-dioxane
SUBSTR	EUH210: Safety data sheet available on request. Preservatives: < 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
STOP	-

Further information can be found in the safety data sheet.

Limitations of the Procedure

Contamination of reagents and samples by bacteria or fungi as well as cross-contamination of the test kit reagents and samples can lead to incorrect results. Incorrect washing to separate unbound

components from the sample and test reagents as well as incorrect incubation times of the samples and controls can cause incorrect results as well.

The overall interpretation of an ELISA test result should consider all clinical findings.

In certain cases testing of another serum sample taken at intervals of a few weeks may be helpful.

Sample Treatment

Specimen materials

Serum of human origin

Sample Shelf Life and Storage

Samples can be stored at 2...8 °C for a maximum of 2 days. For longer periods samples have to be stored at -20 °C. Repeated (> 3x) freezing and thawing of samples should be avoided.

Sample Preparation

Bring samples to room temperature before use. Homogeneity should be ensured by shaking components briefly. Samples have to be diluted 1 : 51 (v/v) with sample diluent.

Example: 10 µL sample and 500 µL sample diluent.

Reagent Treatment

Reagent Shelf Life and Storage

The complete test kit with sealed reagent bottles and microtitration strips can be stored at 2...8 °C until the printed expiration date. All opened test kit components are stable for up to 2 months when stored properly at 2...8 °C. The diluted wash buffer can be stored at 2...8 °C for up to 1 month.

Reagent Preparation

The microtiter plate with breakable 8-well strips is vacuum sealed with desiccant. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored at 2...8 °C and protected from moisture in the original cover carefully resealed.

Dilute wash buffer (10 x) 1 : 10 with deionized water.

Example: 10 mL wash buffer (10 x) + 90 mL deionized water.

Except for the wash buffer all components included in the test kit are ready to use.

Assay Procedure

1. Allow test reagents and required number of wells to reach room temperature (RT). Shake reagents gently before use. Avoid foaming.
2. Pipette 100 µL **DILUENT** into the required number of cavities.
3. Pipette 20 µL **CONTROL +** Positive control
20 µL **CONTROL -** Negative control
20 µL diluted sample each, mix gently.
4. Cover the plate and incubate for 30 min at 37 °C.
5. Decant, then wash each well 5 x with 300 µL diluted wash buffer. Tap dry onto absorbent paper if necessary.
6. 100 µL **CONJ HRP** HRP conjugate per cavity.
7. Cover the plate and incubate for 15 min at 37 °C.
8. Decant, then wash each well 5 x with 300 µL diluted wash buffer. Tap dry onto absorbent paper if necessary.
9. 100 µL **SUBSTR** substrate per cavity.
10. Incubate for 10 min at RT **protected from light**.
11. 100 µL **STOP** stop solution per cavity, mix gently.
12. Read OD at 450 nm and ≥ 620 nm with a microplate reader within 30 min following reaction stop.

Evaluation of Results

Determination of Cut-off

The cut-off is 0.25 absorbance units. The gray zone is defined as the range between 0.8 x cut-off and cut-off value. Serum samples with absorbances above cut-off value are considered reactive for anti-*Francisella tularensis* antibodies, serum samples with absorbances below the gray zone are considered non-reactive. Reactive serum samples should be verified in a second test. A sample with absorbance values in the gray zone should be re-tested with a new serum sample collected at an interval of 1 - 2 weeks.

Interpretation of Results

Negative	< 0.20
Positive	> 0.25
Gray zone	0.20 – 0.25

For samples with results within the gray zone, the test should be repeated.

Test Validation

The test run is valid if

- absorbance of negative control ≤ 0.20
- absorbance of positive control ≥ 1.50

If the above-mentioned quality criteria are not met, the test should be repeated strictly following the Instructions for Use (reagent preparation, incubation times and temperatures, wash steps, etc.). In case of repeated failure of the quality criteria contact the manufacturer.

Performance Characteristics

Sensitivity and Specificity

A panel of 402 serum samples was tested with Serazym® Anti-Francisella tularensis in comparison to an independent, commercially available ELISA.

n = 402	ELISA positive	ELISA negative
Serazym® ELISA positive	89	1
Serazym® ELISA negative	0	312

Sensitivity: 100,0 %

Specificity: 99.7 %

Precision

For the determination of the intra-assay-coefficient of variation (CV) samples were measured in a 12-fold determination in one test run.

Pool of antibody positive sera (titer of predilution)	\bar{x} OD	CV (%)
1:50	2.713	2.6
1:100	2.286	4.3
1:200	1.971	3.3
1:400	1.606	4.3
1:800	1.172	3.2
1:1600	0.800	4.5
1:3200	0.511	6.0
1:6400	0.334	8.6

Application

Automatic Processing

The operator is responsible for the validation of the microtiter plate processors and associated application files before using this product. Application files for the use of the automated microtiter plate processors listed below may be requested from your local distributor.

Performing Serazym® Anti-Francisella tularensis on automated microtiter plate processors (e.g., DS2® and DSX®, Dynex Technologies) may cause increased absorbance values in comparison to the manual processing caused by differences in the wash procedures and technical specifications of the equipment. In these cases, a maximum value of $OD_{450/620\text{ nm}} = 0.3$ is permissible for the negative control. It is recommended to program a wash protocol with 10 s soak time per strip and wash step. A final wash step with deionized water and a soak time of 10 s is recommended after each wash cycle. If necessary, the number of washing steps may be increased to 7x or 8x.

Change History

Version	Section	Modifications
2025-06_v03_de_en	Test Components Important Information Assay Procedure Performance Characteristics Application	Removal of weak control increase of conjugate volume Update of Safety Instructions Inclusion Automatic Processing Removal Titration Curve Layout and editorial changes
2025-08	Assay Procedure English version	Step 2 in Assay Procedure: "WELLS" replaced by "DILUENT" Editorial changes

References

1. Büyüç, F et al. (2016): "The prevalence of tularemia in occupational groups that have contact with animals". Turk J Med Sci, Vol. 46, p. 451-456.
2. Chaignat, V et al. (2014): "Performance of seven serological assays for diagnosing tularemia". BMC Infectious Diseases, vol. 14: 234.
3. Erdem, H et al. (2014): "Evaluation of tularaemia courses: a multicentre study from Turkey". Clin Microbiol Infect, vol. 20, p. 1042-1051.
4. Njeru, J et al. (2017): "Serological evidence of Francisella tularensis in febrile patients seeking treatment at remote hospitals, northeastern Kenya, 2014–2015". New Microbe and New Infect, vol. 19, p. 62–66.
5. Robert Koch Institut (2016): "Tularämie". Epidemiologisches Bulletin Nr. 12
6. Schmitt, P et al (2004): "A novel screening ELISA and a confirmatory Western blot useful for diagnosis and epidemiological studies of tularemia". Epidemiol. Infect., vol. 133, p. 759–766.
7. Yanes, H et al. (2017): "Evaluation of In-House and Commercial Serological Tests for Diagnosis of Human Tularemia". J Clin Microbiol, vol. 56:1.