












# Serazym<sup>®</sup> Verotoxin 1+2

Enzyme immunoassay for the qualitative detection of verotoxins 1 and 2 (shiga toxins 1 and 2) in stool samples of human origin and of enrichment culture (supernatant)

REF E-030 ▽<sub>Σ</sub> 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](mailto:info@seramun.com) • [www.seramun.com](http://www.seramun.com)

<p><span style="border: 1px solid black; padding: 2px;">IVD</span> In-vitro diagnostic medical device</p> <p> Country of manufacture and date of manufacture</p> <p> Keep away from sunlight</p> <p> Consult instructions for use</p> <p> Sufficient for <i>n</i> tests</p>	<p><span style="border: 1px solid black; padding: 2px;">UDI</span> Unique device identifier</p> <p><span style="border: 1px solid black; padding: 2px;">REF</span> Article number</p> <p> Humidity limitation</p> <p> Temperature limit</p> <p> Biohazard</p>	<p> Manufacturer</p> <p><span style="border: 1px solid black; padding: 2px;">SN</span> Serial number</p> <p><span style="border: 1px solid black; padding: 2px;">LOT</span> Batch code</p> <p> Do not reuse</p> <p> Use-by date</p> <p> Attention</p>
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## Intended Use

Serazym® Verotoxin 1+2 is an IVD test for the qualitative determination of verotoxins 1 and 2 (shiga toxins 1 and 2) in stool samples of human origin and of enrichment culture (supernatant) through manual or semi-automatic processing by a laboratory professional user.

It is intended to aid in the diagnosis of an EHEC-associated gastroenteritis in patients with symptoms of a gastroenteritis and for screening of verotoxins 1 and 2 (shiga toxins 1 and 2) in samples of asymptomatic close contacts.

## Principle of the Test

Serazym® Verotoxin 1+2 is an enzyme immunoassay based on polyclonal and monoclonal antibodies against verotoxin 1 (VT1) and 2 (VT2). Supernatants of enrichment culture or diluted, untreated stool samples as well as negative and positive controls samples are dispensed into wells of the microtiter plate coated with polyclonal anti-VT1 and anti-VT2 antibodies. After incubation, unbound components are removed by a washing step. Then, biotinylated monoclonal anti-VT1 and anti-VT2 antibodies are dispensed into the wells. After incubation, unbound components are removed by a washing step, followed by an incubation step with horseradish peroxidase (HRP)-conjugated streptavidin. After incubation and a washing step, HRP converts the colorless substrate solution to a blue reaction product in the following enzymatic reaction step. The reaction is stopped by the addition of stop solution, resulting in a color change from blue to yellow. The optical density (OD) of the reaction product measured at 450 nm measuring filter and  $\geq 620$  nm reference filter, respectively, is directly proportional to the concentration of specifically bound VT1 and VT2 antigens.

## Test Components (Delivery Scope)

		<b>For 96 wells</b>	
1	<b>WELLS</b>	<b>Microtiter plate</b> coated with < 10 µg/mL polyclonal anti-VT1 and anti-VT2 antibodies (sheep)	12 single breakable 8-well strips, orange color marking, vacuum-sealed with desiccant
2	<b>WASHBUF (10x)</b>	<b>Wash buffer (10x)</b> Seramun® Wash buffer A TRIS-based buffer	100 mL concentrate for 1000 mL solution, colorless, white cap
3	<b>DIL</b>	<b>Sample diluent</b> Seramun® Sample diluent G TRIS-based buffer	100 mL, ready to use, colored orange, black cap
4	<b>CONTROL +</b>	<b>Positive control</b> < 1 µg/mL native E. coli-reactive sample (inactivated)	2.0 mL, ready to use, colored blue, red cap
5	<b>CONTROL -</b>	<b>Negative control</b> TRIS-based buffer	2.0 mL, ready to use, colored blue, green cap
6/1	<b>CONJ BIOTIN</b>	<b>Biotin conjugate</b> < 5 µg/mL biotinylated, monoclonal anti-VT1 and anti-VT2 antibodies (mouse)	15 mL, ready to use, colored green, white cap
6/2	<b>CONJ STREPT</b>	<b>Streptavidin-HRP conjugate</b> < 1 µg/mL	15 mL, ready to use, colored red, green cap

7	<b>SUBSTR</b>	<b>Substrate</b> SeramunBlau® automat fast < 0.1 % 3,3',5,5'- tetramethylbenzidine; < 0.05 % hydrogen peroxide	15 mL, ready to use, colorless, blue cap
8	<b>STOP</b>	<b>Stop solution</b> SeramunBlau® stop 0.25 M sulphuric acid	15 mL, ready to use, colorless, yellow cap
9		<b>Certificate of Analysis</b>	1 piece
10		<b>Instructions for Use</b>	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 microtiter plate washer • microtiter plate reader with 450 nm measuring filter and ≥ 620 nm reference filter • deionized water • measuring cylinder • tubes for sample preparation • enrichment broth, e.g., mTryptic-Soy-Broth (mTSB) containing mitomycin C

## Important Information

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

Do not use reagents from damaged packages or bottles. The shelf life specified must be observed. Do not mix components with reagents from other manufacturers.

**Mixing of test kit components of different lots is permitted only for sample diluent, wash buffer, substrate and stop solution. The sample diluent IS NOT universally applicable!**

**Wash buffer, substrate and stop solution are universally applicable for Serazym® stool ELISA Adenovirus (E-017), Astrovirus (E-045), Norovirus (E-061), Rotavirus (E-020), Campylobacter (E-093), Clostridium difficile GDH (E-107), Clostridium difficile Toxin A+B (E-040), Cryptosporidium parvum (E-039), Entamoeba histolytica (E-018), Giardia (E-106) and H. pylori 2nd Gen. (E-114).**

All serious incidents occurring in relation with Serazym® Verotoxin 1+2 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. Bring all test components to room temperature before use. Reagents that appear contaminated should not be used.

Each well of a microtiter plate can be used once only. Each sample and control have 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. Allow the wash buffer to remain in the wells for at least 5 seconds per wash cycle. Remove wash buffer residues by thoroughly aspirating or tapping out the wells!

Protect the substrate from light!



## Safety Instructions


Reagents must not be swallowed. Contact with skin or mucous membranes should be avoided.

Some reagents may contain biocides as preservative.

Handle all components and patient samples as potentially hazardous and infectious.

Additional information may be taken from the Material Safety Data Sheet.

Product contains the following hazardous component/-s:

Test component	Hazard labeling and supplementary information on ingredients
<b>WELLS</b>	Contains material of animal origin.
<b>WASHBUF (10x)</b>	<p>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.</p> <p>EUH210: Safety data sheet available on request.</p> <p>Preservatives: &lt; 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); &lt; 0.1 % 5-bromo-5-nitro-1,3-dioxane</p>
<b>DIL</b>	<p>Contains material of animal origin.</p> <p>Preservatives: &lt; 0.1 % sodium azide</p>
<b>CONTROL +</b>	<p>Contains material of microbial and animal origin.</p> <p>Preservatives: &lt; 0.1 % sodium azide</p>
<b>CONTROL -</b>	<p>Contains material of animal origin.</p> <p>Preservatives: &lt; 0.01 % sodium azide</p>
<b>CONJ BIOTIN</b>	<p>EUH210: Safety data sheet available on request.</p> <p>Contains material of animal origin.</p> <p>Preservative: &lt; 0.0015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1); &lt; 0.1 % 5-bromo-5-nitro-1,3-dioxane</p>
<b>CONJ STREPT</b>	<p>EUH210: Safety data sheet available on request.</p> <p>Contains material of animal origin.</p> <p>Preservative: &lt; 0.01 % 5-bromo-5-nitro-1,3-dioxane</p>
<b>SUBSTR</b>	<p>Hazard component: 2-Pyrrolidone</p> <p>Signal word: Danger</p>  <p>H360: May damage fertility or the unborn child.</p> <p>P201: Obtain special instructions before use.</p> <p>P280: Wear protective gloves/protective clothing/eye protection/face protection.</p> <p>P308+P313: IF exposed or concerned: Get medical advice/attention.</p> <p>Restricted to professional users.</p> <p>Preservatives: &lt; 0.00015 % reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)</p>
<b>STOP</b>	-

### Limitations of the Procedure

The qualitative enzyme immunological detection of VT1 and VT2 does not allow for a correlation between measured OD and severity of infection. The OD of the specimen is not to be correlated with the OD of the positive control. Cross contamination of reagents and samples may result in false positive results. Incorrect dilutions, insufficiently homogenized samples, and particles not sedimented by centrifugation may cause false positive as well as false negative test results. A negative test result obtained with Serazym® Verotoxin 1+2 does not exclude an EHEC infection. False negative tests may result from improper timing of sample collection or inhomogeneous antigen distribution in the sample. Due to generally low toxin concentrations in stool samples, the selective enrichment of EHEC bacteria in specific culture media has a positive effect on the sensitivity of toxin detection by Serazym® Verotoxin 1+2. The overall interpretation of the ELISA test result should consider the full clinical picture. Individual cases may require retesting at intervals of several weeks.

## Sample Treatment

### Sample Collection

Collect stool sample in suitable sampling container.

Example: Stool collection tube, with spoon, screw cap, (LxØ): 107 x 25 mm, transparent

### Sample Shelf Life and Storage

Untreated stool samples should be stored at 2...8 °C or at -20 °C immediately after collection and analyzed within 72 hours.

The culture supernatant after enrichment can be stored for up to 5 days at 2...8 °C, at room temperature, or at -20 °C. Repeated (>3 times) freezing and thawing of the culture supernatant after enrichment should be avoided due to the risk of erroneous results. Culture supernatants after enrichment that have already been diluted in Seramun® Sample Diluent G according to the instructions for use can be stored for up to 5 days at 2...8 °C and subsequently analyzed by ELISA.

### Sample Preparation

#### Sample preparation for testing of enrichment cultures

Mix untreated stool samples well. Transfer 100 to 200 mg or 100 to 200 µL stool sample into a tube with 4.5 mL enrichment medium, e.g. mTryptic-Soy-Broth (mTSB) containing Mitomycin C and incubate for 18 to 20 h at 37°C (98°F). If possible, use a shaker for sample incubation.

**Caution:** Please observe the specifications of the manufacturer of the enrichment medium.

Due to potential inhomogeneous distribution of verotoxin in the matrix, sampling of two different points in a stool sample is recommended. Allow floating particles to sediment or sediment suspended particles by centrifugation in a microcentrifuge for 1 min at maximum speed. Dilute the culture supernatant 1 : 2 with sample diluent. Pipette 100 µL sample diluent into a reaction tube. Transfer 100 µL of the culture supernatant into the sample diluent and mix thoroughly.

#### Sample preparation for testing of diluted stool specimens

See section *Application*

## 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

Microtiter plate with breakable 8-well strips is vacuum sealed with desiccant. Allow packaging to reach room temperature before opening. Protect unused wells from moisture and store refrigerated with desiccant in the original bag carefully resealed. Dilute wash buffer (10x) 1 : 10 with deionized water. Example: 10 mL Seramun® Wash buffer A (10x) + 90 mL deionized water.

## 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 120 µL **CONTROL +** Positive control  
120 µL **CONTROL -** Negative control  
100 µL diluted stool specimen or supernatants of enrichment culture.
3. Cover the plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap dry onto absorbent paper if necessary.
5. Add 120 µL **CONJ BIOTIN** biotin conjugate per well.
6. Cover plate and incubate for 30 min at RT.
7. Decant, then wash each well 5x with 300 µl diluted wash buffer.  
Tap the plate dry on absorbent paper if necessary.
8. Add 120 µL **CONJ STREPT** streptavidin-HRP conjugate per well.
9. Cover plate and incubate for 30 min at RT.
10. Decant, then wash each well 5x with 300 µl diluted wash buffer.  
Tap the plate dry on absorbent paper if necessary.
11. Add 120 µL **SUBSTR** substrate per well.
12. Incubate for 15 min at RT protected from light.
13. Add 120 µL **STOP** stop solution per well, mix gently.
14. Read OD at 450 nm measuring and ≥ 620 nm reference filter with a microtiter plate reader within 30 min following reaction stop.

## Evaluation of Results

### Qualitative Evaluation:

Cut-off determination: OD negative control + 0.20

Samples showing OD values equal to or higher than the cut-off are considered positive. Samples with OD values below cut-off are considered negative for verotoxin 1 and 2 antigens.

The test run is valid if

- the mean OD value of the negative control is  $\leq 0.20$  (manual processing)  
 $\leq 0.30$  (automatic processing)
- the mean OD value of the positive control is  $\geq 1.00$

If the above-mentioned quality criteria are not met, test should be repeated strictly following the test procedure (incubation times and temperatures, sample and wash buffer dilution, wash steps, etc.). In case of repeated failure of the quality criteria contact the manufacturer.

## Interpretation of Results

Positive	$\geq$ cut-off
Negative	$<$ cut-off

It is recommended that each laboratory establishes its own normal and pathological reference ranges.

## Performance Characteristics

### Precision

To determine precision, 4 stool samples with varying verotoxin 2 contents were measured multiple times. For the determination of the intra-assay-coefficient of variation (CV) samples were measured in a 12-fold determination in one test run. The determination of the inter-assay-coefficient of variation was done by a 3-fold determination in 11 different test runs.

Verotoxin 2 (pg/mL)	Intra-assay-coefficient of variation		Inter-assay-coefficient of variation	
	$\bar{x}$ OD	CV (%)	$\bar{x}$ OD	CV (%)
3125	2.268	2.3	2.026	2.8
800	0.799	4.7	0.752	7.3
200	0.262	5.1	0.241	9.3
0	0.056	11.3	0.048	15.1

### Detection Limit

The lower detection limit of the Serazym® Verotoxin 1+2 was determined at  $< 100$  pg/mL ( $< 10$  pg/well) by separate titration of purified VT1 and VT2.

## Sensitivity and Specificity

Sensitivity and specificity of Serazym® Verotoxin 1+2 were determined in a retrospective study in comparison to a vero-cell cytotoxicity test with 825 stool samples.

The cohort included 795 stool samples to be tested for pathogenic intestinal pathogens and 30 stool samples pre-characterized by shiga toxin gene PCR and culture and stored at -20 °C until testing. ELISA testing was performed after pre-enrichment of the samples (mTSB; 18 – 20 h at 37 °C).

n = 30		Vero-cell cytotoxicity assay	
		positive	negative
Serazym® ELISA	positive	11	0
	negative	2	17

Sensitivity: 84.6 %

n = 795		Vero-cell cytotoxicity assay	
		positive	negative
Serazym® ELISA	positive	0	2
	negative	0	793

Specificity: 99.8 %

## Cross Reactivity

Routine microbiological diagnostics of the stool samples included in the clinical study revealed 141 samples positive for a pathogen originating from 12 different bacterial species:

*Staphylococcus aureus*, enterotoxin positive and negative *E. coli* strains (EHEC); *Clostridium difficile* toxin-positive strains, *Pseudomonas aeruginosa*; *Salmonella spp.* (e.g., *Salmonella typhimurium*; *Salmonella enteritidis*); *Aeromonas hydrophila*; *Aeromonas caviae*; *Campylobacter spp.*; *Hafnia alvei*; *Yersinia enterocolitica* O:3.

None of these pathogens caused false positive results in the Serazym® Verotoxin 1+2.

## Application

### Sample Preparation

#### Sample preparation for direct testing from diluted stool specimens

Mix untreated stool samples well and dilute 1 : 3.5 with sample diluent. Pipette 500 µL sample diluent into a reaction tube. For solid or semi-solid stool samples transfer 200 mg (approx. 4 – 6 mm diameter) with a disposable stick, for liquid stool samples transfer 200 µL into the sample diluent and mix thoroughly. If necessary, sediment suspended particles by centrifugation in a microcentrifuge for 1 min at maximum speed. Due to possible inhomogeneous verotoxin distribution, sampling of two different positions of a stool sample is recommended.

**Caution:** The direct use of stool samples in the Serazym® Verotoxin 1+2 ELISA without prior enrichment may be considered as a preliminary screening to obtain preliminary results. Subsequent testing of the samples after enrichment should be performed in addition to obtain a sufficiently high sensitivity.

**A negative ELISA result does not exclude an infection with EHEC when testing stool samples without enrichment culture.**

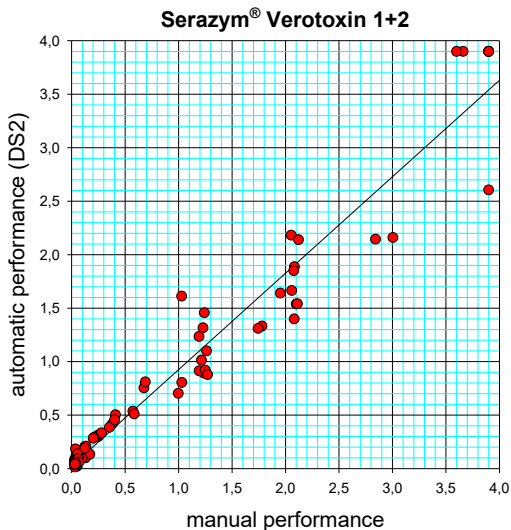
### Automatic Processing

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® Verotoxin 1+2 on fully automated microplate processors (e.g. DS2®, DSX®, Dynex Technologies or ThunderBolt®, Gold Standard Diagnostics) may cause elevated absorbance values in comparison to the manual procedure caused by differences in the wash procedures and technical specifications of the equipment. In these cases, a maximum value of OD = 0.3 is permissible for the negative control. It is recommended to program a wash protocol with at least 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.

### Correlation: manual – automatic processing

A panel of 188 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at  $r = 0.98$ .



## Change History

<b>Version</b>	<b>Section</b>	<b>Modifications</b>
2026-04	Cover sheet	Adjustment of REF number to packaging concept
	Test Components (Delivery Scope)	Adjustment of volumes to packaging concept, addition of quantity or concentration of the active ingredient
	Additional Materials and Aids Required for the Test Procedure	Addition of "reagent container for multi-channel micropipettes"
	Important information	Table under "Safety instructions": Adjustment to the labeling on the label
	Sample Treatment	Addition of sample vessel example; Addition of data on culture growth to the "Sample Shelf Life and Storage" section
	Assay Procedure	Adaptation to packaging concept
2026-05	Application: Automatic Processing	Addition of user responsibility for the validation of microtiter plate processors