














# Serazym<sup>®</sup> Entamoeba histolytica

Enzyme immunoassay for the qualitative detection of *Entamoeba histolytica*-specific proteins in stool samples of human origin

<b>REF</b>	E-018		96
<b>IVD</b>	In-vitro-diagnostic medical device		<b>CE</b>

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<b>IVD</b> In-vitro diagnostic medical device	<b>UDI</b> Unique device identifier	 Manufacturer
 Country of manufacture and date of manufacture	<b>REF</b> Article number	<b>SN</b> Serial number
 Keep away from sunlight	 Humidity limitation	<b>LOT</b> Batch code
 Consult instructions for use	 Temperature limit	 Do not reuse
 Sufficient for <i>n</i> tests	 Biohazard	 Use-by date
		 Attention

## Intended Use

Serazym® *Entamoeba histolytica* is an IVD test for the qualitative determination of *Entamoeba histolytica* specific proteins in stool samples of human origin through manual or semi-automatic processing by a laboratory professional user.

It is intended to aid in the diagnosis of amoebiasis in samples from patients with symptoms of gastroenteritis and to the monitoring of an infection with *E. histolytica* during an effective treatment with antibiotics.

## Principle of the Test

Serazym® *Entamoeba histolytica* is an enzyme immunoassay based on monoclonal and polyclonal antibodies recognizing different epitopes of the serine-rich 30 kDa membrane protein (SREHP) of *Entamoeba histolytica*. Diluted, untreated stool samples as well as negative and positive controls are dispensed into wells of the microtiter plate coated with a mixture of monoclonal and polyclonal anti-SREHP antibodies. After incubation, unbound components are removed by a washing step and peroxidase (HRP)-labeled anti-SREHP antibodies are dispensed into the wells. After incubation, unbound components are removed by a washing step, then HRP converts the colorless substrate solution to a blue reaction product in the following enzymatic reaction step. After incubation 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 filter and  $\geq 620$  nm reference filter is directly proportional to the concentration of the specifically bound *Entamoeba histolytica* antigen.

## Test Components (Delivery Scope)

		For 96 wells	
1	<b>WELLS</b>	<b>Microtiter plate</b> coated with $< 5 \mu\text{g/mL}$ monoclonal (mouse) and polyclonal (rabbit) anti-SREHP antibodies	12 single breakable 8-well strips brown 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 A Phosphate-based buffer	100 mL, ready to use, colored yellow, black cap
4	<b>CONTROL +</b>	<b>Positive control</b> $< 1 \mu\text{g/mL}$ synthetic <i>E. histolytica</i> antigen	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	<b>CONJ HRP</b>	<b>HRP-conjugate</b> $< 5 \mu\text{g/mL}$ HRP-labeled mixture of monoclonal (mouse) and polyclonal (sheep) anti-SREHP antibodies	15 mL, ready to use, colored green, 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

## 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 wash buffer (10x), sample diluent, negative control, substrate and stop solution.**

**Wash buffer (10x), sample diluent, negative control, 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® Entamoeba histolytica 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 only be used once. Each sample and control should be pipetted with a new pipette tip. Positive and negative control 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 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. Handle all components and patient samples as potentially hazardous and infectious. Additional information may be taken from the 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 HRP</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 *Entamoeba histolytica* antigens in stool samples does not allow a correlation between the measured OD and the severity of an infection. Also, it is not allowed to correlate absorbances of samples with the absorbance of the positive control.

Cross contamination of reagents and samples may result in false results. Incorrect dilutions, insufficiently homogenized samples, and particles not sedimented by centrifugation may cause false negative as well as false positive test results. A negative test result in Serazym® *Entamoeba histolytica* does not exclude an *Entamoeba histolytica* infection, because the number of excreted antigens may fall below the detection limit in invasive amoebiasis samples. Thus, additional investigations (e.g., other methods to detect specific antibodies or ultrasound) should be performed in case of a negative ELISA result but clinical suspicion. The overall interpretation of the ELISA test result should consider the full clinical picture. Individual cases may require repeated testing 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

Stool samples should be stored at 2...8 °C immediately after collection and examined within 72 h or stored frozen at -20 °C. Repeated freezing (> 3x) and thawing of samples should be avoided due to the risk of incorrect results. Stool samples that have already been diluted in Seramun® Sample diluent A according to the instructions for use can be stored at 2...8 °C for up to 72 h and subsequently analyzed by ELISA.

### Sample Preparation

Mix untreated stool samples well and dilute 1 : 6 with sample buffer.

Example: Pipette 500 µL sample buffer into a reaction tube. For solid or semi-solid stool samples transfer 100 mg (approx. 2 - 3 mm diameter) with a disposable stick, for liquid stool samples transfer 100 µL into the sample buffer and mix thoroughly. If necessary, sediment suspended particles by centrifugation in a microcentrifuge for 1 min at maximum speed.

Preserved stool samples or stool samples stored in transport media should not be used in Serazym® *Entamoeba histolytica*.

## 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 wash buffer (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 100 µL **CONTROL +** Positive control  
100 µL **CONTROL -** Negative control  
100 µL diluted stool specimen each.
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 100 µL **CONJ HRP** HRP 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 on absorbent paper if necessary.
8. Add 100 µL **SUBSTR** substrate per well.
9. Incubate for 10 min at RT **protected from light**.
10. Add 100 µL **STOP** stop solution per well, mix gently.
11. Read OD at 450 nm measuring filter and  $\geq 620$  nm reference filter with a microplate 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 *Entamoeba histolytica* 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 of value the positive control is  $\geq 0.80$

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, 3 samples were measured multiple times. For the determination of the intra-assay coefficient of variation (CV), the samples were measured in a 40-fold determination in one test run. The determination of the inter-assay coefficient of variation was done by a 2-fold determination in a total of 40 test runs, spread over 20 days and 2 operators:

Sample	Intra-assay coefficient of variation		Inter-assay coefficient of variation	
	$\bar{x}$ OD	CV (%)	$\bar{x}$ OD	CV (%)
1	2.022	2.7	1.448	9.2
2	1.106	2.1	0.883	7.6
3	0.413	4.9	0.374	9.4

### Detection Limit

The lower limit of detection of Serazym® *Entamoeba histolytica* has been determined by titration of stool samples spiked with *E. histolytica* HM-1:IMSS (ATCC 30459) trophozoites with < 150 trophozoites/mL.

### Sensitivity and Specificity

A collective of n = 188 stool samples of human origin (from a microbiological routine laboratory) was examined in Serazym® *Entamoeba histolytica*:

Negative: n = 178

Positive: n = 10

Specificity: 94.7 %

In the reference test the 10 samples tested positive in the ELISA were confirmed as *Entamoeba histolytica*-negative and a specificity of 94.7 % could be confirmed.

Pre-characterized *Entamoeba histolytica*-positive stool samples of human origin were processed with Serazym® *Entamoeba histolytica* and compared to a commercially available ELISA.

n = 39	ELISA positive	ELISA negative
<b>Serazym® ELISA positive</b>	14	5**
<b>Serazym® ELISA negative</b>	2*	18

Sensitivity: 87.5 %

Specificity: 78.3 %

Samples labeled with \* and \*\* were re-tested by an external laboratory using PCR antigen detection. This results in a corrected sensitivity of 100 % and a corrected specificity of 81.8 %.

## Cross reactivity

Negative stool suspensions were spiked with the following microorganisms with a bacterial count of  $\geq 10^8$  colony-forming units (cfu) per mL in sample buffer and tested negative in the Serazym® *Entamoeba histolytica* (450 nm measuring filter and  $\geq 620$  nm reference filter < cut-off):

<i>Aeromonas hydrophila</i>	ATCC (7966)	<i>Campylobacter coli</i>	ATCC (33559)
<i>Bacillus cereus</i>	ATCC (11778)	<i>Campylobacter jejunii</i>	ATCC (32291)
<i>Bacillus subtilis</i>	ATCC (6633)	<i>Campylobacter fetus</i>	ATCC (27374)
<i>Bacteroides fragilis</i>	ATCC (25285)	<i>Campylobacter upsaliensis</i>	ATCC (43954)
<i>Citrobacter freundii</i>	ATCC (8090)	<i>Campylobacter lari</i>	ATCC (35221)
<i>Clostridium sordelli</i>	ATCC (9714)	<i>Vibrio cholerae</i>	clinical isolate
<i>Enterobacter aerogenes</i>	ATCC (13048)	<i>Yersinia enterocolitica</i> O:3	clinical isolate
<i>Enterobacter cloacae</i>	ATCC (13047)	<i>Yersinia enterocolitica</i> O:9	clinical isolate
<i>Enterococcus faecalis</i>	ATCC (29212)	<i>Yersinia enterocolitica</i> Y11	clinical isolate
<i>Escherichia coli</i>	ATCC (25922)	<i>Yersinia enterocolitica</i> RKI 0803733	clinical isolate
<i>Klebsiella pneumoniae</i>	ATCC (13883)	<i>Clostridium difficile</i>	VPI 10463
<i>Peptostreptococcus anaerobius</i>	ATCC (27337)	<i>Salmonella infantis</i>	ATCC (51741)
<i>Proteus vulgaris</i>	ATCC (8427)	<i>Salmonella anatum</i>	ATCC (9270)
<i>Pseudomonas aeruginosa</i>	ATCC (10145)	<i>Salmonella paratyphi</i> A	ATCC (11511)
<i>Salmonella enterica</i> Serovar <i>typhimurium</i>	ATCC (14028)	<i>Salmonella paratyphi</i> B	ATCC (8759)
<i>Salmonella enterica</i> ssp. <i>enteritidis</i>	ATCC (13076)	<i>Salmonella paratyphi</i> C	Nr. 2 Pasteur
<i>Shigella flexneri</i>	ATCC (12022)	<i>Lactococcus lactis</i>	DSM (20481)
<i>Shigella sonnei</i>	ATCC (25931)	<i>Proteus mirabilis</i>	ATCC (29906)
<i>Staphylococcus aureus</i>	ATCC (25923)	<i>Pseudomonas fluorescens</i>	ATCC (13525)
<i>Staphylococcus epidermidis</i>	ATCC (12228)	<i>Pseudomonas putida</i>	ATCC (49128)
<i>Vibrio parahaemolyticus</i>	ATCC (17802)	<i>Streptococcus agalactiae</i>	ATCC (13813)
<i>Candida albicans</i>	ATCC (10231)	<i>Morganella morganii</i>	ATCC (25830)

Fecal samples containing *Entamoeba dispar* in high concentrations (at least  $3.9 \cdot 10^5$  cfu/mL sample) may result in a positive signal in the Serazym® *Entamoeba histolytica*.

## Interference

None of the following substances in the indicated concentrations added to *Entamoeba histolytica* positive and negative stool samples showed a significant impact on the test result:

(-)-Scopolamine N-butyl bromide (0.5 %, Buscopan®), barium sulfate (5 %), bismuth(III) subsalicylate (0.5 %, Pepto-Bismol), Cyclamat (5 %), Diclofenac (0.5 %), hemoglobin human (5 %), blood human (5 %), Hylak® N (5 %), Iberogast® (5 %), Imodium® akut duo (0.06/3.8 %), Loperamid hydrochloride (5 %, Loperamid-CT akut), Metronidazole (0.5 %), Mucin (5 %), Nexium® (0.06 %), Nifuroxazide (0.5 %, Pentofuryl®), palmitic acid (20 %), Perenterol forte (0.5 %), Rennie® (20 %), Simagel® (1 %), stearic acid (20 %), Vancomycin (0.5 %).

## Application

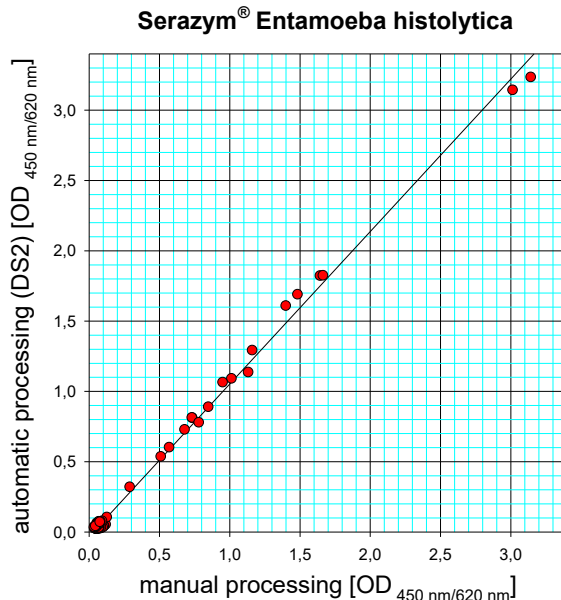
### Automatic processing

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® *Entamoeba histolytica* on fully automated microplate processors (e.g., DS2®, DSX®; Dynex Technologies) 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 wash steps may be increased to 7x or 8x.

### Correlation: manual – automatic processing

A panel of 140 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at  $r = 0.998$ .



## Change History

Version	Section	Modifications
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	Addition of negative control as a component across lots and products; Table under "Safety instructions": Adjustment to the labeling on the label
	Sample Treatment	Addition of sample vessel example
	Assay Procedure	Adaptation to packaging concept
2026-05	Application: Automatic Processing	Addition "user responsibility"