















# Serazym<sup>®</sup> Cryptosporidium parvum

Enzyme immunoassay for the qualitative detection of *Cryptosporidium parvum*-specific antigens in stool samples of human origin

|            |                                    |   |    |
|------------|------------------------------------|---|----|
| <b>REF</b> | E-039                              |  | 96 |
| <b>IVD</b> | In-vitro-diagnostic medical device |  |    |



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|  |  |  |
|--|--|--|
| <p><b>IVD</b> 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><b>UDI</b> Unique device identifier</p> <p><b>REF</b> Article number</p> <p> Humidity limitation</p> <p> Temperature limit</p> <p> Biohazard</p> | <p> Manufacturer</p> <p><b>SN</b> Serial number</p> <p><b>LOT</b> Batch code</p> <p> Do not reuse</p> <p> Use-by date</p> <p> Attention</p> |
|--|--|--|

## Intended Use

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

It is intended to aid in the diagnosis of cryptosporidiosis in samples of patients with symptoms of a gastroenteritis and for screening of *Cryptosporidium parvum*-specific antigens in samples of asymptomatic close contacts.

## Principle of the Test

Serazym® *Cryptosporidium parvum* is an enzyme immunoassay based on monoclonal antibodies against *Cryptosporidium (C.) parvum*-specific antigens. Diluted, untreated stool samples as well as negative and positive controls are dispensed into the wells of the microtiter plate coated with monoclonal anti-*C. parvum* antibodies. After incubation, unbound components are removed by a washing step and peroxidase (HRP)-labeled monoclonal anti-*C. parvum* 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 specifically bound *Cryptosporidium parvum* antigens.

## Test Components (Delivery Scope)

|   |                      | <b>For 96 wells</b>  |   |
|---|----------------------|--|---|
| 1 | <b>WELLS</b>         | <b>Microtiter plate</b><br>coated with < 5 µg/mL<br>monoclonal anti- <i>C. parvum</i><br>antibodies (mouse)                | 12 single breakable<br>8-well strips,<br>black color marking,<br>vacuum-sealed with desiccant |
| 2 | <b>WASHBUF (10x)</b> | <b>Wash buffer (10x)</b><br>Seramun® Wash buffer A<br>TRIS-based buffer  | 100 mL concentrate for 1000 mL<br>solution, colorless,<br>white cap                           |
| 3 | <b>DIL</b>           | <b>Sample diluent</b><br>Seramun® Sample diluent A<br>Phosphate-based buffer   | 100 mL, ready to use,<br>colored yellow,<br>black cap   |
| 4 | <b>CONTROL +</b>     | <b>Positive control</b><br>< 1% native <i>C. parvum</i> -reactive<br>sample (inactivated)                                  | 2.0 mL, ready to use,<br>colored blue,<br>red cap   |
| 5 | <b>CONTROL -</b>     | <b>Negative control</b><br>TRIS-based buffer   | 2.0 mL, ready to use,<br>colored blue,<br>green cap   |
| 6 | <b>CONJ HRP</b>      | <b>HRP conjugate</b><br>< 5 µg/mL HRP-labeled,<br>monoclonal anti- <i>C. parvum</i><br>antibodies (mouse)                  | 15 mL, ready to use,<br>colored green,<br>green cap   |
| 7 | <b>SUBSTR</b>        | <b>Substrate</b><br>SeramunBlau® automat fast<br>< 0.1 % 3,3',5,5'-<br>tetramethylbenzidine;<br>< 0.05 % hydrogen peroxide | 15 mL, ready to use,<br>colorless,<br>blue cap  |
| 8 | <b>STOP</b>          | <b>Stop solution</b><br>SeramunBlau® stop<br>0.25 M sulphuric acid   | 15 mL, ready to use,<br>colorless,<br>yellow cap  |

|    |                         |         |
|----|-------------------------|---------|
| 9  | Certificate of Analysis | 1 piece |
| 10 | 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 reservoirs for multi-channel pipetting • 8-channel wash comb with vacuum pump and waste bottle or microplate washer • microplate reader with 450 nm measuring filter and  $\geq$  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 laboratory 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<sup>®</sup> 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<sup>®</sup> Cryptosporidium parvum 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 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. 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 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 *Cryptosporidium parvum* 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 sample absorbance values 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 obtained with Serazym® *Cryptosporidium parvum* does not exclude a *Cryptosporidium parvum* infection. False negative tests may result from inhomogeneous antigen distribution in the sample. For this reason, it is recommended to repeat the test with a new stool sample, if a patient has been tested negative, but an infection is suspected. 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.

Stool samples that are already diluted in transport media (PARA-PAK PLUS, PARA-PAK PLUS SAF) may result in decreased OD values in Serazym® *Cryptosporidium parvum* in comparison to untreated samples. In case of samples with low antigen concentrations OD values may drop below the detection limit of the test. Therefore, it is recommended to use untreated samples. If untreated samples are not available, prediluted samples should be diluted less than stated below.

## 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 immediately after collection at 2...8 °C or -20 °C and examined within 72 h. Repeated (> 3x) freezing 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 72 h and subsequently analyzed by ELISA.

Stool samples preserved in transport media (PARA-PAK PLUS, PARA-PAK PLUS SAF) may also be tested in Serazym® *Cryptosporidium parvum*.

### 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.

Mix stool samples preserved in transport media (PARA-PAK PLUS, PARA-PAK PLUS SAF) well and test in Serazym® *Cryptosporidium parvum* without further dilution.

## 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  $\mu\text{L}$  **CONTROL +** Positive control  
100  $\mu\text{L}$  **CONTROL -** Negative control  
100  $\mu\text{L}$  diluted stool specimen each.
3. Cover the plate and incubate for 60 min at RT.
4. Decant, then wash each cavity 5x with 300  $\mu\text{L}$  diluted wash buffer.  
Tap dry onto absorbent paper if necessary.
5. Add 100  $\mu\text{L}$  **CONJ HRP** HRP conjugate per well.
6. Cover the plate and incubate for 30 min at RT.
7. Decant, then wash each cavity 5x with 300  $\mu\text{L}$  diluted wash buffer.  
Tap the plate dry on absorbent paper if necessary.
8. Add 100  $\mu\text{L}$  **SUBSTR** substrate per well.
9. Incubate for 10 min at RT **protected from light**.
10. Add 100  $\mu\text{L}$  **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 + 0.10

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 *Cryptosporidium parvum* 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  $\geq 0.80$

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, 4 stool samples were measured multiple times. For the determination of the intra-assay coefficient (CV) of variation samples were measured in an 8-fold determination in one test run. The determination of the inter-assay coefficient of variation was done by a 2-fold determination in 8 different test runs.

| Sample | Intra-assay coefficient of variation |        | Inter-assay coefficient of variation |        |
|--------|--------------------------------------|--------|--------------------------------------|--------|
|        | $\bar{x}$ OD                         | CV (%) | $\bar{x}$ OD                         | CV (%) |
| 1      | 1.732                                | 4.98   | 1.704                                | 3.89   |
| 2      | 0.855                                | 11.14  | 0.969                                | 10.21  |
| 3      | 0.697                                | 5.54   | 0.519                                | 4.45   |
| 4      | 0.129                                | 4.33   | 0.159                                | 7.67   |

### Detection Limit

The lower limit of detection of Serazym® *Cryptosporidium parvum* was determined at  $1.6 \times 10^5$  oocysts per mL by titration of oocysts in a negative stool sample.

### Sensitivity and Specificity

A collective of  $n = 327$  stool samples of human origin (from a routine microbiological laboratory) was examined in Serazym® *Cryptosporidium parvum*:

Negative:  $n = 325$

Positive:  $n = 2$

Specificity: 99.4 %

The two ELISA positive samples were confirmed to be positive by direct immunofluorescence:

Specificity corrected: 100 %

*Cryptosporidium parvum* positive pre-characterized stool samples of human origin were investigated by Serazym® *Cryptosporidium parvum* in comparison to two commercially available ELISA.

| n = 34                         | ELISA 1 positive | ELISA 1 negative |
|--------------------------------|------------------|------------------|
| <b>Serazym® ELISA positive</b> | 32               | 0                |
| <b>Serazym® ELISA negative</b> | 1*               | 1                |

Sensitivity compared to ELISA 1: 96.9%

\* The Serazym® ELISA negative and ELISA 1 positive sample were confirmed to be negative by direct immunofluorescence test.

| n = 19                         | ELISA 2 positive | ELISA 2 negative |
|--------------------------------|------------------|------------------|
| <b>Serazym® ELISA positive</b> | 18               | 0                |
| <b>Serazym® ELISA negative</b> | 0                | 1                |

Sensitivity compared to ELISA 2: 100%

## Cross Reactivity

Stool samples positive for one of the following pathogens have been tested with Serazym® *Cryptosporidium parvum* and did not show any cross reactivity:

Adenovirus, *Ancylostoma duodenale*, *Ascaris lumbricoides*, astrovirus, *Blastocystis hominis*, *Campylobacter jejuni*, *Clostridioides difficile*, *Entamoeba dispar / histolytica*, *Giardia lamblia*, *Helicobacter pylori*, norovirus, rotavirus, *Salmonella* spp.

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

|                               |              |
|-------------------------------|--------------|
| <i>Aeromonas hydrophila</i>   | (ATCC 7966)  |
| <i>Bacillus cereus</i>        | (ATCC 11778) |
| <i>Bacillus subtilis</i>      | (ATCC 6633)  |
| <i>Bacteroides fragilis</i>   | (ATCC 25285) |
| <i>Candida albicans</i>       | (ATCC 10231) |
| <i>Campylobacter coli</i>     | (ATCC 33559) |
| <i>Campylobacter jejuni</i>   | (ATCC 33291) |
| <i>Citrobacter freundii</i>   | (ATCC 8090)  |
| <i>Clostridium sordellii</i>  | (ATCC 9714)  |
| <i>Enterobacter aerogenes</i> | (ATCC 13048) |
| <i>Enterobacter cloacae</i>   | (ATCC 13047) |
| <i>Enterococcus faecalis</i>  | (ATCC 29212) |
| <i>Escherichia coli</i>       | (ATCC 25922) |

|   |                   |
|---|-------------------|
| <i>Klebsiella pneumoniae</i>                    | (ATCC 13883)      |
| <i>Peptostreptococcus anaerobius</i>            | (ATCC 27337)      |
| <i>Proteus vulgaris</i>                         | (ATCC 8427)       |
| <i>Pseudomonas aeruginosa</i>                   | (ATCC 10145)      |
| <i>Salmonella enterica serovar enteritidis</i>  | (ATCC 13076)      |
| <i>Salmonella enterica serovar typhimurium</i>  | (ATCC 14028)      |
| <i>Shigella flexneri</i>                        | (ATCC 12022)      |
| <i>Shigella sonnei</i>                          | (ATCC 25931)      |
| <i>Staphylococcus aureus</i>                    | (ATCC 25923)      |
| <i>Staphylococcus epidermidis</i>               | (ATCC 12228)      |
| <i>Vibrio parahaemolyticus</i>                  | (ATCC 17802)      |
| <i>Vibrio cholerae</i>                          | Clinical isolate  |
| <i>Yersinia enterocolitica serotypes O3, O9</i> | Clinical isolates |

## Interference

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

Barium sulfate (5 %), Buscopan® (2 mg/mL), Cyclamate (5 %), Diclofenac (2 mg/mL), human hemoglobin (5 mg/mL), human blood (5 %), Hylak® N (5 %), Iberogast® (5 %), Imodium® akut duo (0.2 / 12.5 mg/mL), Loperamide (0.2 mg/mL), Metronidazole (2 mg/mL), Mucin (5 mg/mL), Nexium® (2 mg/mL), palmitic acid (20 %), Pentofuryl® (2 mg/mL), Pepto-Bismol (1 mg/mL), Perenterol (2.5 mg/mL), Rennie® (8 mg/mL), Simagel® (2 mg/mL), stearic acid (20 %), Vancomycin (2 mg/mL).

## Application

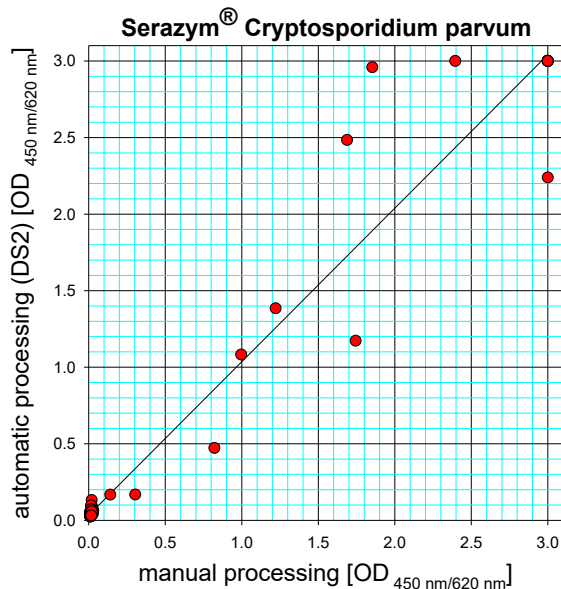
### Automatic Processing

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® *Cryptosporidium parvum* 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. If necessary, the number of wash steps may be increased to 7x or 8x.

### Correlation: manual – automatic processing

A panel of 90 stool specimens was processed manually and automatically in parallel (DS2®, Dynex Technologies). The correlation coefficient was calculated at  $r = 0.976$ .



## 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 negative control as a component across lots and products; 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   |
|                | Assay Procedure   | Adaptation to packaging concept   |
| 2026-05        | Application: Automatic Processing                             | Addition of user responsibility for the validation of microtiter plate processors   |