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DENARASE ELISA Kit Instruction Leaflet
c-LEcta GmbH
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DENARASE ELISA Kit

ELISA Kit for the determination of *Serratia marcescens* endonuclease in process-derived samples.

For research and manufacturing use only.

INTENDED USE

This ELISA kit is intended for the quantitative in vitro measurement of *Serratia marcescens* endonuclease. This test may be used to monitor the removal of endonucleases such as DENARASE® or Benzonase® Nuclease from process-related samples for process development and quality control purposes. **The kit is for research and manufacturing use only and is not intended for diagnostic procedures.**

TEST PRINCIPLE

This DENARASE ELISA kit is a sandwich ELISA to be performed in a microtest plate format. Samples potentially containing *Serratia marcescens* endonuclease are incubated in microtest plate wells which have been pre-coated with a specific monoclonal capture antibody alongside a standard curve of different endonuclease concentrations. After incubation and a washing step in which unbound components are removed, a biotin-conjugated, specific monoclonal detector antibody is added. After further washing steps, the bound detector antibody reacts with an enzyme conjugate acting as tracer. After a final washing step, a substrate solution is added to the wells and reacted, resulting in color development. The optical density is measured photometrically and is proportional to the analyte concentration present in the wells. The endonuclease concentration in unknown samples can be calculated based on the corresponding DENARASE standard curve.

REAGENTS AND MATERIALS PROVIDED IN THE KIT

No.	Reagent	Details	Quantity
(1)	Microtest plate (ready to use)	96 well (12 strips of 8 wells), pre-coated with a monoclonal antibody specific for *Serratia marcescens* endonuclease	5 microplates
(2)	Washing and dilution buffer (10×)	Tris buffered saline containing surfactant and preservative, 10× concentrate	3 × 100 mL
(3)	Washing and dilution buffer (10×)	Tris buffered saline containing surfactant and preservative, 10 × concentrate	1 × 20 mL
(4)	DENARASE Standard (10 µg/mL)	DENARASE Endonuclease in a buffered solution with preservative	1 × 0.1 mL
(5)	Detector antibody (100×)	Monoclonal antibody specific for *Serratia marcescens* endonuclease, conjugated to biotin, in a buffered solution with preservative, 100× concentrate	1 × 0.75 mL
(6)	Enzyme conjugate (100×)	Streptavidin-conjugated horseradish peroxidase in stabilized solution, 100× concentrate	1 × 0.75 mL
(7)	Substrate solution	TMB One Substrate solution, ready to use	1 × 75 mL
(8)	Stop solution	0.5 M sulfuric acid	1 × 75 mL

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED WITH THE KIT

- Ultrapure water (at least double distilled quality) for dilution of the washing buffer and the dilution buffer (supplied as 10× concentrate)
- Absorbent paper towels for removing residual liquid after microtest plate washing
- Suitable test tubes for preparing standards, controls and samples
- Suitable containers for washing and dilution buffer
- Suitable reagent reservoirs for effective multi-channel pipetting

- Lid for covering the microtest plates during the incubation steps
- Orbital microtest plate shaker (about 500 rpm) and vortex mixer
- Microtest plate washer (manual washing can alternatively be performed)
- Precision pipettes (adjustable volumes from 10 µL to 5000 µL) with suitable tips
- Multi-channel pipette (100 µL) with suitable tips
- Microplate reader capable of measuring optical density at 450 nm (reference wavelength of 620 – 690 nm)

STORAGE OF THE REAGENTS

All supplied reagents should be kept refrigerated at 2 – 8°C and should be used before their expiration dates.

WARNINGS AND PRECAUTIONS

- This kit is intended for research and manufacturing only and should only be used by qualified personnel.
- Read the instruction leaflet carefully before starting the assay.
- Note lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
- Follow good laboratory practice and safety guidelines. Wear lab coats, disposable gloves and protective glasses when necessary.
- Some reagents of the kit contain Proclin®300 as preservative. These reagents may cause eye and skin irritations and should be handled with care. In case of contact with eyes or skin, flush immediately with water.
- Store the substrate solution protected from light.
- The stop solution consists of 0.5 molar sulfuric acid. This reagent is corrosive and may cause eye and skin irritations. It should be handled with care. In case of contact with eyes or skin, flush immediately with water.
- Remaining kit reagents and prepared solutions should be treated as potentially hazardous.
- Waste according to national safety guidelines and regulations.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.

Preparation of the Washing Buffer (1×)

Dilute the washing buffer 10× (2) 1:10 with ultrapure water prior to use (e.g. 100 mL 10 × concentrate with 900 mL ultrapure water). The washing buffer (1×) is stable at room temperature and may be stored for up to two weeks.

Preparation of the Dilution Buffer (1×)

Dilute the 10× concentrate buffer (3) 1:10 with ultrapure water prior to use (e.g. 10 mL 10× concentrate with 90 mL ultrapure water). The dilution buffer (1×) is stable at room temperature and may be stored for up to two weeks.

Preparation of DENARASE Standards

The standard concentrations for the DENARASE ELISA should be prepared from the DENARASE Standard (4) supplied with the kit immediately before performing the assay. The standards should be used only on the day of preparation.

DENARASE ELISA standards (PD1 and S1 to S8) are prepared in dilution buffer (1×) according to the following dilution scheme:

Standard ID	Concentration [pg/ml]	Volume Antigen [µL]		Volume Dilution Buffer [µL]
		µl	of	
DENARASE Standard	10.000.000	–	–	–
PD1	100.000	10	DENARASE Standard	990
S1	1.000	20	PD1	1.980
S2	500	1.000	S1	1.000
S3	250	1.000	S2	1.000
S4	125	1.000	S3	1.000
S5	62,5	1.000	S4	1.000
S6	31,25	1.000	S5	1.000
S7	15,625	1.000	S6	1.000
S8	0	–	–	1.000

PD: Pre-Dilution; S: Standard

Preparation of the Detector Antibody Working Solution (1×)
Dilute the detector antibody 100× concentrate (5) 1:100 with dilution buffer (1×). For one microtest plate, 120 µL of the 100× concentrate are mixed with 12 mL dilution buffer. This working solution should be used only on the day of preparation.

Preparation of the Enzyme Conjugate Working Solution (1×)
Dilute the enzyme conjugate 100× concentrate (6) 1:100 with dilution buffer (1×). For one microtest plate, 120 µL of the 100× concentrate are mixed with 12 mL dilution buffer. This working solution should be used only on the day of preparation.

SAMPLE PREPARATION

- Any aggregates present in the samples should be removed by centrifugation to ensure proper assay performance.
- In general, all sample working dilutions should only be used on the day of preparation.
- Samples should be diluted with dilution buffer (1×) prior to the assay. The minimum sample dilution should be 1:2.

ASSAY PROCEDURE

All ELISA steps are performed at room temperature (18 – 26°C).
Allow all materials and reagents of the kit to reach room temperature before use.
Do not open the foil bag of the pre-coated microtest plate (1) before reaching room temperature.
Remaining plate strips not used in the assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.
During all incubation steps the plate should be covered with a lid (not provided with the kit) to prevent evaporation and contamination of solutions.

1. Incubation with standards and samples:
Fill the plate with 100 µL/well of DENARASE standards and assay controls as well as the diluted samples and incubate for 1 hour with continuous shaking at 500 rpm. Standards, assay controls and samples should be analyzed at least in duplicates. Triplicates are recommended.

2. Washing:
Remove the contents of the wells and wash the plate 4 × with 250 µL/well of washing buffer (1×). After washing, invert the plate to decant the contents of the wells. Discard any residual liquid by blotting and firmly tapping against clean absorbent paper.

3. Incubation with detector antibody:
Fill the plate with 100 µL/well of the detector antibody working solution (1×) and incubate 0.5 hours with continuous shaking.

4. Washing:
Remove the contents of the wells and wash the plate 4 × with 250 µL/well of washing buffer (1×). After washing, invert the plate to decant the contents of the wells. Discard any residual liquid by blotting and firmly tapping against clean absorbent paper.

5. Incubation with enzyme conjugate:
Fill the plate with 100 µL/well of the enzyme conjugate working solution (1×) and incubate 20 minutes with continuous shaking.

6. Washing:
Remove the contents of the wells and wash the plate 4 × with 250 µL/well of washing buffer (1×). After washing, invert the plate to decant the contents of the wells. Discard any residual liquid by blotting and firmly tapping against clean absorbent paper.

7. Incubation with substrate solution and stopping:
Add 100 µL/well of the substrate solution (7) and incubate the plate for 15 min with continuous shaking. If color development is too low after 15 minutes the substrate incubation can be extended up to 30 minutes. Stop the color reaction by adding directly 100 µL/well of the stop solution (8) resulting in a yellow colored product.

8. Measurement:
Measure the optical density at 450 nm (OD450) with a multichannel microplate reader. A reference wavelength between 620 nm and 690 nm is recommended.

CALCULATIONS

Average the replicate OD450 values for each standard, control and sample.

Create a standard curve with the aid of a suitable software, using preferably the nonlinear regression mode of the four- or five-parameter equation.

The concentration of unknown samples calculated from the calibration curve must be multiplied by the dilution factor of the sample.

ELISA PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The limit of detection (LOD), defined as the lowest endonuclease concentration that could be differentiated from the assay blank, was calculated to ~4 pg/ml. The limit of detection was determined by calculating the endonuclease concentration that corresponds to an OD450 value of three standard deviations above the mean OD450 of the assay blank.

The limit of quantitation (LOQ) was confirmed at a concentration corresponding to the threefold LOD . The LOQ is 12 pg/ml.

Working Range

The working range of the DENARASE ELISA was defined between ~32 pg/ml and 1000 pg/ml of endonuclease.

Note: Based on data generated in October 2018.

TROUBLESHOOTING

Possible reasons for insufficient assay performance are listed below.

A. No reactivity throughout the whole plate

- Omission of an incubation step or a reagent
- Use of reagents in the wrong order
- Inadequate preparation of ELISA components/-reagents

B. Poor reactivity throughout the whole plate

- Inadequate storage or preparation of ELISA components/-reagents
- Reagents were not allowed to reach room temperature before use
- Improper wavelength for measuring the optical density

C. Reactivity and/or assay background too high throughout the whole plate

- Improper washing step(s)
- Inadequate storage or preparation of ELISA components/-reagents
- Overincubation of reagents, for example incubation with substrate solution before stopping

D. Poor Intra-Assay Precision (CV of replicates too high)

- Improper washing step(s)
- Insufficient mixing of standards, samples and assay control samples
- Inappropriate sample preparation (debris or aggregates in the samples disturb the assay performance by increasing the imprecision and should be removed by centrifugation)

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Benzonase® Nuclease is a registered trademark of Merck KGaA