Endonuclease ELISA

Manual

ELISA kit for quantitative *in vitro* measurement of endonuclease produced by *Serratia marcescens*.

Catalog No.:	PRNUC
Contents:	1 x 96 well plate
Storage conditions:	2–8°C
Version:	02

For research use only.

PROGEN

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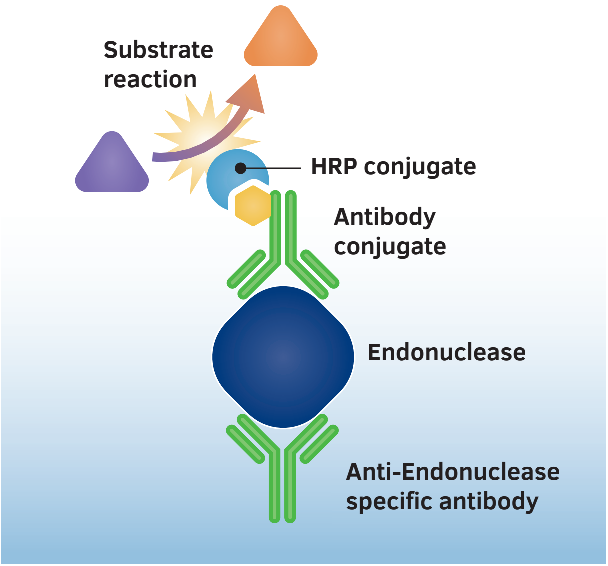
1. Introduction

Endonucleases are enzymes that cleave the phosphodiester bonds within a nucleic acid strand, cutting DNA or RNA at specific internal sites. In the context of viral gene therapy, the detection and quantification of endonucleases are critical for ensuring the safety and efficacy of the final gene therapy product. Endonucleases, often used during the manufacturing process to remove unwanted nucleic acids, must be thoroughly removed or minimized to prevent unintended degradation of the vector genome.

This ELISA kit is intended for the quantitative *in vitro* measurement of an endonuclease of *Serratia marcescens*. This assay may be used to monitor the presence of endonucleases such as DENARASE® or Benzonase® in process-related samples during process development and quality control. The assay includes the enzyme DENARASE® as a standard to generate the standard curve. When measuring different endonucleases with this assay, for accurate quantification, correction factors may be applied or custom enzyme standards may be used. The kit is for research use only and is not intended for diagnostic procedures.

2. Test Principle

This Endonuclease ELISA kit is a sandwich ELISA to be performed in a microplate format. A specific monoclonal capture antibody is coated onto wells of a microplate and is used to capture endonuclease from *Serratia marcescens* present in samples, e.g. cell lysates or purified virus preparations. Captured endonucleases are detected in two steps:



1. A biotin-conjugated monoclonal detector antibody (antibody conjugate) binds to the immobilized endonuclease.

2. A streptavidin peroxidase conjugate (HRP conjugate) binds to the biotin molecules.

Adding the substrate solution (TMB) results in a color reaction, which is proportional to the analyte concentration present in the wells. The absorbance is measured photometrically at an optical density of 450 nm (reference wavelength between 620 – 690 nm). The endonuclease concentration in unknown samples can be calculated based on the corresponding DENARASE® standard curve.

3. Test Kit Contents

Endonuclease microplate	96 well (12 strips of 8 wells), pre-coated with a monoclonal antibody specific for the endonuclease of *Serratia marcescens*, ready to use	1 microplate
Buffer B02 (10x)	Tris buffered saline containing surfactant and preservative, 10x concentrate, for washing and dilution	3 x 24 ml
DENARASE® standard (10 µg/ml)	DENARASE® endonuclease in a buffered solution containing a preservative	20 µl
Antibody conjugate (100x)	Monoclonal antibody specific for *Serratia marcescens*, conjugated to biotin, in a buffered solution containing a preservative, 100x concentrate	1 x 150 µl
HRP conjugate (100x)	Streptavidin-conjugated horseradish peroxidase in stabilized solution, 100x concentrate	1 x 150 µl
TMB	TMB ONE substrate solution, ready to use	1 x 15 ml
STOP	0.5 M sulfuric acid, ready to use	1 x 15 ml
Adhesive foil		2

4. Required Material

Ultrapure water (at least double distilled quality) for dilution of the buffer B02 (provided as 10x concentrate)
Absorbent paper towels for removing residual liquid after microplate washing via tapping
Test tubes for preparing standards, controls and samples
Containers for the diluted buffer B02 (1x)
Reagent reservoirs for multi-channel pipetting
Orbital microplate shaker (about 500 rpm) and vortex mixer
Microplate washer (manual washing can alternatively be performed)
Precision pipettes (adjustable volumes from 10 µl to 5000 µl) and pipette tips
Multi-channel pipette with pipette tips
Microplate reader capable of measuring optical density at 450 nm (reference wavelength between 620 – 690 nm)

5. Preparation of Reagents

Bring all reagents and samples to room temperature before use.

Buffer B02 (10x) (wash and dilution buffer)

1. Dilute the buffer B02 (10x) 1:10 with ultrapure water prior to use (e.g. 20 ml 10x concentrate with 180 ml ultrapure water).
2. The diluted component is named buffer B02 (1x). The buffer B02 (1x) is stable at 2 – 8 °C and may be stored for up to two weeks.

DENARASE® standard

DENARASE® standards (PD and S1 to S7 and blank) are prepared in buffer B02 (1x).

The standard should be prepared immediately before performing the assay. The standard should be used only on the day of preparation.

Name	Dilution	Concentration	Volume antigen			Volume buffer B02 (1x)
		ng/ml	µl			µl
PD	1:100	100	10	DENARASE® standard	+	990
S1	1:100	1	20	PD	+	1980
S2	1:2	0.5	1000	S1	+	1000
S3	1:2	0.250	1000	S2	+	1000
S4	1:2	0.125	1000	S3	+	1000
S5	1:2	0.063	1000	S4	+	1000
S6	1:2	0.031	1000	S5	+	1000
S7	1:2	0.015	1000	S6	+	1000
Blank	0	0	-	-		1000

S, Standard; PD, Pre-Dilution

Example for a plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Sp1	Sp1								
B	S1	S1	Sp2	Sp2								
C	S2	S2	Etc.	Etc.								
D	S3	S3										
E	S4	S4										
F	S5	S5										
G	S6	S6										
H	S7	S7										

S, Standard; Sp1, Specimen dilution 1; Sp2, Specimen dilution 2

Antibody conjugate (100x)

1. Dilute the Antibody conjugate (100x) 1:100 with buffer B02 (1x) and mix with a roller mixer. For one microplate, 120 µl of the 100x concentrate are mixed with 11.88 ml buffer B02 (1x).
2. The diluted component is named Antibody conjugate (1x). This working solution should be used only on the day of preparation.

HRP conjugate (100x)

1. Dilute the HRP conjugate (100x) 1:100 with buffer B02 (1x) and mix with a roller mixer. For one microplate, 120 µl of the 100x concentrate are mixed with 11.88 ml buffer B02 (1x).
2. The diluted component is named HRP conjugate (1x). This working solution should be used only on the day of preparation.

6. Storage of the Reagents

All supplied reagents should be kept refrigerated at 2–8°C and should be used before their expiration dates. Remaining plate strips not used in the assay should be repacked in the bag with desiccant. Close the bag tightly for refrigerated storage.

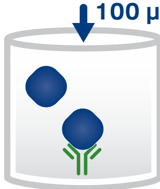
7. Sample Preparation

- Samples should be diluted with buffer B02 (1x) prior to the assay. The minimum sample dilution should be 1:2.
- In general, all sample working dilutions should only be used on the day of preparation.
- Any aggregates present in the samples should be removed by centrifugation to ensure proper assay performance.

8. Short Protocol

1 DENARASE® dilutions
Sample dilutions
assay controls

100 µl

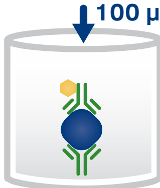


1 hour
RT
500 rpm

4 times 250 µl buffer B02 (1x)

2 Antibody conjugate (1x)

100 µl

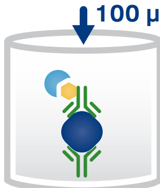


30 min
RT
500 rpm

4 times 250 µl buffer B02 (1x)

3 HRP conjugate (1x)

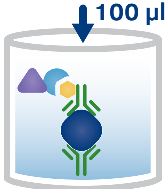
100 µl



20 min
RT
500 rpm

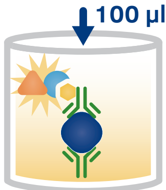
4 times 250 µl buffer B02 (1x)

4 **TMB**



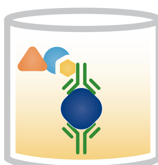
15 min
RT
500 rpm

5 **Stop**



RT

6 **Read at 450 nm (and 620 – 690 nm)**



Read within 30 min

9. Test 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 microplate before reaching room temperature.

During all incubation steps the plate should be covered with an adhesive foil to prevent evaporation and contamination of solutions.

1. **Incubation with standards and samples:** Add 100 µl/well of the working dilutions of DENARASE® standards, internal assay controls as well as the diluted samples and blank to the microplate and incubate for 1 hour with continuous shaking at 500 rpm. Standards, internal assay controls and samples should be analyzed at least in duplicates. Triplicates are recommended.

   Washing: Remove the liquid from the wells. For washing, fill each well with 250 µl of buffer B02 (1x), incubate approximately 5 sec, discard and tap inverted plate onto clean absorbent paper. Carry out four washing steps in total.

2. **Incubation with Antibody conjugate (1x):** Fill the plate with 100 µl/well of the Antibody conjugate (1x). Seal strips with adhesive foil and incubate 30 min with continuous shaking at 500 rpm.

   Washing: Remove the liquid from the wells. For washing, pipette 250 µl/well of buffer B02 (1x), incubate approximately 5 sec, discard and tap inverted plate onto clean absorbent paper. Carry out four washing steps in total.

3. **Incubation with HRP conjugate (1x):** Fill the plate with 100 µl/well of the HRP conjugate (1x). Seal strips with adhesive foil and incubate 20 min with continuous shaking at 500 rpm.

   Washing: Remove the liquid from the wells. For washing, pipette 250 µl/well of buffer B02 (1x), incubate approximately 5 sec, discard and tap inverted plate onto clean absorbent paper. Carry out four washing steps in total.

4. **Incubation with TMB:** Add 100 µl/well of TMB. Seal strips with adhesive foil and incubate the plate for 15 min with continuous shaking at 500 rpm. If color development is too low after 15 min the substrate incubation can be extended up to 30 min.

5. **STOP color reaction:** Stop the color reaction by adding directly 100 µl/well of STOP solution resulting in a yellow colored liquid.

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

10. Calculation of Results

Average the replicate OD450 values for each standard, control and sample. If applicable, subtract values measured between 620 – 690 nm reference wavelength from values at 450 nm.

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.

11. Test Validity

Parameter	Specification
Mean OD450 Blank	≤ 0.2
Mean OD450 S1	1.5 – 3.0

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

Working Range

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

13. General Information

For professional use.

Release Notes

The instruction manual is only valid in combination with the lot-specific documents (Quality Control Certificate), which are enclosed in each kit.

Please make sure to use the instruction manual with the version number that corresponds to the number on the lot-specific documents. Do not use expired reagents.

Precautions

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.

STOP (sulphuric acid) may cause skin or eye irritation. In the event of eye contact, rinse out immediately with plenty of water and consult a physician!

Remaining kit reagents and prepared solutions should be treated as potentially hazardous.

Safety data sheet is available on request.

Disposal

Product: Chemicals and biological materials must be disposed of in compliance with the respective national regulations.

Packaging: Packaging must be disposed of in compliance with the respective national regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified otherwise, non-contaminated packaging may be treated like household waste or may be recycled.

Transport Damages

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use damaged components for test procedure. Such components or kits should be stored at 2 – 8°C until the complaint is handled.

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