Datasheet for ABIN7505815

AccuSignal™ Nuclease ELISA Kit for Impurity Detection

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Overview

Quantity:	1 kit
Target:	Nuclease
Host:	Rabbit
Clonality:	Polyclonal
Method Type:	Sandwich ELISA
Detection Range:	0.03 ng/mL - 20 ng/mL
Minimum Detection Limit:	0.03 ng/mL
Application:	ELISA, Impurity Detection (Imp De)

Product Details

Purpose:	Nuclease ELISA kit is designed for the quantitative detection of Nuclease/NucA in serum, plasma, and hybridoma cell supernatants.
Brand:	AccuSignal™
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Benzonase, Nuclease, Endonuclease, Serratia marcescens endonuclease, NucA, Denarase
Sensitivity:	3 ng/mL
Components:	This kit contains: Nuclease antibody coated 96-well strip plate, Plate Sealer, Nuclease standard, biotinylated Detection Antibody, streptavidin-peroxidase conjugate, along with buffers and protocol.

Product Details

Material not included:	• Microplate shaker (up tp 450 rpm) • Interval timer • Multichannel pipettor (50-300 µL) • Precision single pipettes (10 µL, 35 µL, 100 µL, 1000 µL, etc.) • Disposable pipette tips • Deionized water • Disposable microcentrifuge Tube(s) or microplate • Polypropylene centrifuge tubes (15 mL) • Spectrophotometer microplate reader (450 nm absorbance, 630–650 nm reference filter) • Disposable gloves • Graduated cylinder • Reagent reservoirs • Vortex mixer • Stir plate & magnetic stir bar • Absorbent paper

Target Details

Target:	Nuclease
Alternative Name:	nucA (Nuclease Products)
Background:	Nucleases are secreted by Serratia marcescens into the medium it surrounds. The enzyme is a sugar-nonspecific hydrolase, capable of cleaving both RNA and DNA in either double or single stranded form. It requires divalent cations, preferably Mg2+, and is functional across a broad pH range from 6 to 10 (optimal at 8-8.5) and wide temperature ranges between 35°C and 44°C. The ability of Serratia to secrete nuclease appears to be regulated. Bacterial cultures at differing cell densities display different kinetics and efficiencies of nuclease secretion [i.e. growth medium, growth conditions, and host cell mutations]. Anti-Nuclease/NucA Antibody is useful for researcher interested in identifying nucleic acid contamination.

Application Details

Application Notes:	This kit was shown to positively detect commercially available nuclease variants, such as Benzonase, Denarase and other analogs.
Assay Time:	3 h
Reagent Preparation:	Preparation of Standards & Test Samples 1. Reconstitute the standard vial with 1.0 mL deionized water to obtain a final concentration of 200 ng/mL. Note: This is the “reference stock solution” that will be used below to make the standards.

2. Prepare dilutions of standard.
3. Test samples should be diluted in Nuclease Kit Sample Buffer based on empirically determined criteria for each sample.

Detection antibody working solution preparation

1. Add 110 µL of conjugated antibody to 11 mL of Sample Buffer for use in a full 96-well assay.
2. Mix well by pipette or inversion. Do not vortex.
3. Distribute antibody working solution as described in the assay procedure.

Note: Volumes may be adjusted so long as final working concentration remains as specified.

Streptavidin-HRP Working Solution Preparation

1. Add 110 µL of Streptavidin-HRP to 11 mL of Sample Buffer, respectively for use in a full 96-well assay.
2. Mix well by pipette or inversion. Do not vortex.
3. Distribute Streptavidin-HRP working solution as described in the assay procedure.

Note: Volumes may be adjusted so long as final working concentration remains as specified

Wash Buffer (1X) Preparation

1. Add 50 mL of Nuclease Kit Wash Buffer (10X) to 450 mL of deionized water.
2. Mix for at least 10-minutes using a magnetic stir bar.

Note: The wash buffer (1X) can be stored at room temperature (15°C to 25°C) for up to 2-weeks, after which it should be discarded.

Assay Procedure:

1. To each well add 100 µL of unknown or standard sample per well and incubate at room temperature for 60-minutes with shaking at 450 revolutions per minutes (rpm) on a shaker.
2. Wash the wells with Wash Buffer as follows:
   - Decant the contents of the wells manually with a hard, rapid downward motion. Fluid should be captured in a receptable designed to collect the waste. Remove all residual reagent from the microplate by tapping it on absorbent paper with the opening facing downwards.
   - Fill each well with 300 µL of Washing Buffer with a multichannel pipettor.
   - Decant the Washing Buffer from the wells with a hard, rapid downward motion. Remove all residual solution from the microplate by tapping it on absorbent paper with the opening facing downwards.
   - Repeat steps ii and iii two more times (total of 3 washings). Do not leave any residual moisture in the wells on each washing step.
3. To each well add 100 µL of detection antibody working solution
4. Incubate at room temperature for 60-minutes, covered to protect from light, with shaking at 450 rpm.

5. Following 60-minute incubation, wash with Wash Buffer as follows:
   - Decant the contents of the wells manually with a hard, rapid downward motion. Fluid should be captured in a receptable designed to collect the waste. Remove all residual reagent from the microplate by tapping it on absorbent paper with the opening facing downwards.
   - Fill each well with 300 µL of Washing Buffer with a multichannel pipettor.
   - Decant the Washing Buffer from the wells with a hard, rapid downward motion. Remove all residual solution from the microplate by tapping it on absorbent paper with the opening facing downwards.
   - Repeat steps ii and iii two more times (total of 3 washings). Do not leave any residual moisture in the wells on each washing step.
6. To each well add 100 µL of Streptavidin-HRP working solution
7. Incubate at room temperature for 20-minutes, covered to protect from light, with shaking at 450 rpm.
8. Following 20-minute incubation, wash with Wash Buffer as follows:
   - Decant the contents of the wells manually with a hard, rapid downward motion. Fluid should be captured in a receptable designed to collect the waste. Remove all residual reagent from the microplate by tapping it on absorbent paper with the opening facing downwards.
   - Fill each well with 300 µL of Washing Buffer with a multichannel pipettor.
   - Decant the Washing Buffer from the wells with a hard, rapid downward motion. Remove all residual solution from the microplate by tapping it on absorbent paper with the opening facing downwards
   - Repeat steps ii and iii two more times (total of 3 washings). Do not leave any residual moisture in the wells on each washing step.
9. Next add 100 µL per well of room temperature TMB solution and incubate the plate with TMB solution at room temperature for 20 minutes (covered and protected from light).
10. Then add 100 µL of stop solution per well. Gently tap the plate to mix, ensuring no bubbles are formed, and read plate within 5 minutes after stopping the reaction.
11. On plate reader, measure absorbance at 450 nm with the reference wavelength set at 630–650 nm.

Calculation of Results:

Follow the steps below to estimate the nuclease concentration of the test samples.

1. Calculate the relative OD 450 using the following formula: Relative OD 450 = (OD 450 of well) – (OD 630-650 nm of the well)
2. Calculate the mean relative OD 450 of the replicates for each standard solution.
3. Plot the standard solutions data as mean relative OD 450 for each standard solution (Y) vs the respective concentration of the standard solutions (X).
4. Fit the standard solution data with a 4-parameter logistic (4-PL) curve. Weight by 1/Y^2 is intended to be used during generation of 4-PL curve
5. Estimate the Nuclease concentration of each test sample well using interpolation from the standard curve. Calculate the average of each respective sample solution concentration.

Note: If the assay samples are from dilutions, multiply the concentrations obtained from

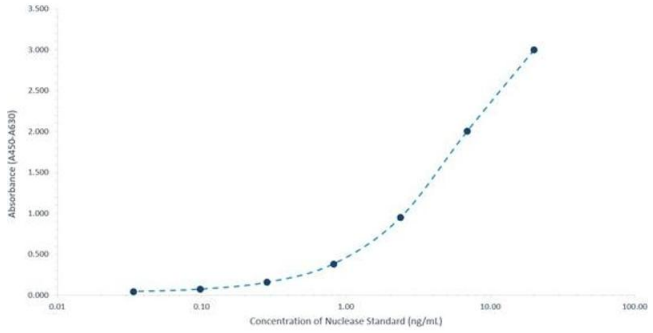
Application Details

	interpolations by the dilution factor. Note: If the spectrometer used for the assay does not automatically subtract the reference wavelength, do this manually.
Assay Precision:	Intra-and inter-assay CV% <20%
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	The kit and reagents should be stored at 2-8°C. Allow reagents to reach room temperature (18-26°C) before use and may be used until the expiration date. It is recommended to aliquot the reconstituted standard solution and store it at -20°C to avoid freeze/thaw cycles.

Images



ELISA

Image 1. The AccuSignal™ Nuclease ELISA Kit has a broad dynamic range that reduces the number of plates and time needed for experiments. The protein standard from the AccuSignal™ Nuclease ELISA Kit was used to prepare a standard curve from 20 to 0.03 ng/mL. The standard curve exhibits a broad range (0.03 to 20 ng/mL) and a strong goodness of fit (r2 ≥ 0.99).