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ab234056
DNase I Assay Kit (Fluorometric)

For the quantitative evaluation of DNase I activity of purified enzymes and their inhibitors as well as comparative examination of DNase I activity in biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

DNase I Assay Kit (Fluorometric) (ab234056) allows for quantitative evaluation of DNase I activity of purified enzymes and their inhibitors as well as comparative examination of DNase I activity in biological samples. Enzyme activity is detected upon cleavage of a DNA Probe, which yields a fluorescent DNA product measured at Ex/Em = 651/681 nm. The limit of quantification (L.O.Q) is 178 fmoles of DNA probe cleaved per minute per mL.

Prepare samples, controls and standards.

Add Reaction Mix to each well containing Positive Control, Test Samples, and Background Control. Add DNA Probe I/DNA Probe Standard Reaction Mix to each well containing DNA Probe I/DNA Probe Standard.

Measure fluorescence (Ex/Em = 651/681 nm) in kinetic mode every 30 seconds for at least 90 minutes at 37°C.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X DNAse I Assay Buffer/10X DNase I Assay Buffer	1.1 mL	-20°C	4°C
DNA Probe I/DNA Probe	1 vial	-20°C	-20°C
DNA Probe Resuspension/DNA Probe Re-suspension Buffer	250 µL	-20°C	RT
DNAse I Positive Control/DNase I Positive Control	1 vial	-20°C	-20°C
Positive Control Resuspension Buffer/Positive Control Re-suspension Buffer	1 mL	-20°C	-20°C
Molecular Biology Grade Water	25 mL	-20°C	RT

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well white plate with flat bottom, low-medium binding.
- Spectrophotometer.
- Purified DNase I, DNase I inhibitors, biological samples.
- Optional: 50 mM 2-Nitro-5-thiocyanatobenzoic acid.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 10X DNAse I Assay Buffer/10X DNase I Assay Buffer

Warm to room temperature before use. Store at 4°C.

5.2 DNA Probe I/DNA Probe

Reconstitute with 220 µL of DNA Probe Resuspension Buffer/DNA Probe Re-suspension Buffer. Aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

5.3 DNA Probe Resuspension Buffer/DNA Probe Re-suspension Buffer

Ready to use as supplied. Store at room temperature.

5.4 DNase I Positive Control

Reconstitute with 220 µL of Positive Control Resuspension Buffer/Positive Control Re-suspension Buffer. Aliquot and store at -20°C.

5.5 Positive Control Resuspension Buffer/Positive Control Re-suspension Buffer

Ready to use as supplied. Store at -20°C.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare 1 µM DNA Probe I/DNA Probe stock by diluting 4 µL of 25 µM DNA Probe I/DNA Probe in 96 µL of molecular biology grade water.
2. Add 0, 4, 8, 12, 16, 20 µL of 1 µM DNA Probe I/DNA Probe into a series of wells on a 96-well plate to generate 0, 4, 8, 12, 16, 20, pmol/well of DNA Probe Standard. Adjust the volume to 50 µL with molecular biology grade water.

   Δ Note: It is imperative to use molecular biology grade water for sample preparation and filter tips for sample pipetting at all times to avoid DNAse contamination.

7. Sample Preparation

1. Thaw any purified enzymes and biological samples.
2. Dilute enzymes, inhibitors, and biological samples to a desired concentration with water or their corresponding storage buffer. Add a desired amount of enzyme, inhibitor, or biological sample to each well and adjust the volume to 50 µL with water. Mix well.

   Δ **Note**: It is imperative to use molecular biology grade water for sample preparation and filter tips for sample pipetting at all times to avoid DNAse contamination.
   Δ **Note**: Do not store enzyme/inhibitor/sample dilutions; discard the dilutions instead.
   Δ **Note**: The recommended amount of serum sample to use in the assay is 10-25 µL.
   Δ **Note**: For uncharacterized enzymes, we suggest testing several doses to ensure the reading is within the Standard Curve range.
   Δ **Note**: If the user suspects any non-specific sample DNase activity, 50 mM 2-Nitro-5-thiocyanatobenzoic acid can be used to specifically inhibit DNase I activity.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

  Δ Note: It is imperative to use molecular biology grade water for sample preparation and filter tips for sample pipetting at all times to avoid DNAse contamination.

8.1 Background Control and Positive Control:

1. Use water only (no enzyme/sample) for background control reaction.
2. For positive control reaction, add 2 µL of DNase I Positive Control to 48 µL of water. Mix well.

8.2 Reaction Mix:

1. Prepare 50 µL of Sample Reaction Mix and DNA Probe Standard Reaction Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Sample Reaction Mix (µL)	DNA Probe Standard Reaction Mix (µL)
10X DNAse I Assay Buffer/10X DNase I Assay Buffer	10	10
DNA Probe I/DNA Probe (25 µM)	2	-
DNase I Positive Control	-	2
Molecular Biology Grade H_2O	38	38

2. Add 50 µL of the Sample Reaction Mix to each well containing the Positive Control, Test Samples, and Background Control. Add 50 µL of DNA Probe Standard Reaction Mix to each well containing DNA Probe Standard.

8.3 Measurement:

1. For positive control, test samples, background control, and DNA Probe Standard measure fluorescence (Ex/Em = 651/681 nm) in kinetic mode every 30 seconds for at least 90 minutes at 37°C.
2. Adjust GAIN/PMT setting of your fluorometer as necessary so that the standard curve readings are within the detection range of the instrument.

9. Data Analysis

9.1 Standard Curve:

1. Record RFU at t = 90 min for each DNA Probe standard curve reading.
2. Plot the DNA Probe standard curve with pmol of DNA on the x-axis and RFU on the y-axis.
3. Apply a linear fit to the DNA standard values and determine the standard curve equation.

9.2 Samples/Positive Control:

1. Subtract background control readings from samples.
2. Apply RFU values at each time point to the standard curve equation to determine pmol of DNA cleaved at each reaction time point.
3. Plot pmol DNA on the y-axis vs. time (in minutes) on the x-axis and determine the slope (pmol/min) of the linear portion of the reaction curve.

$$\text{Sample DNAse I Activity} = \frac{\text{Slope}}{V} * D = \ = \text{pmol/min/mL} \ = \ \mu\text{U/mL}$$

$$\text{Sample Specific Activity} = \frac{\text{Slope}}{\mu g} * D = \ = \text{pmol/min/}\mu\text{g} \ = \ \mu\text{U/}\mu\text{g}$$

Where:

V = sample volume added in the sample wells [mL].

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

Slope = pmol/min (from the linear range of the activity curve).

Unit Definition: One unit of DNase I is the amount of enzyme that cleaved 1.0 µmol of DNA Probe per min. at 37°C.

10. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only.**

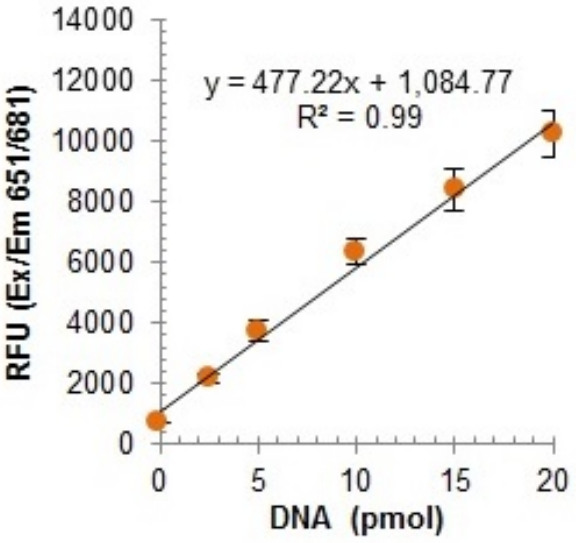

Figure 1. DNA Probe to Product conversion standard curve.

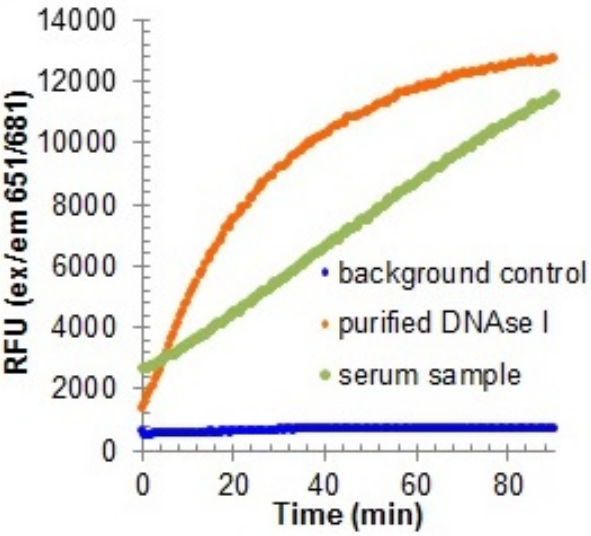

Figure 2. Representative activity curve for purified DNase I (orange), serum sample (green), and background control (blue) at 37°C.

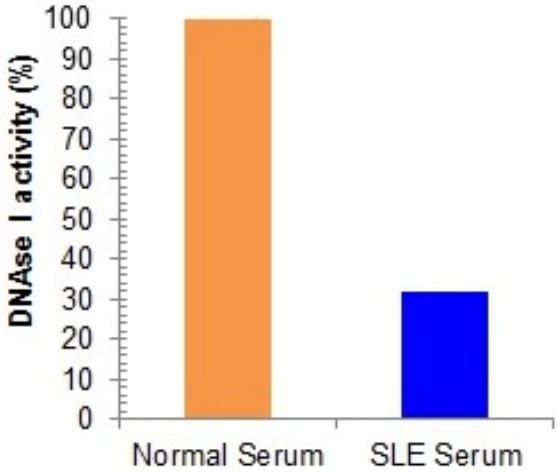

Figure 3. Comparative analysis of DNase I activity from 25 µL undiluted single donor normal vs. Systematic Lupus Erythematosus (SLE) patient serum sample.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)