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DNAse I Activity Assay Kit (Fluorometric)

(Catalog # K429-100; 100 assays; Store at -20°C)

I. Introduction:

Deoxyribonuclease I (DNAse I) is an endonuclease that cleaves DNA phosphodiester bonds yielding 5′-phosphorylated and 3′-hydroxylated oligonucleotides. DNAse I targets single-stranded DNA, double-stranded DNA, and chromatin in a non-specific manner. As an important player in cellular waste management, DNAse I is normally secreted extracellularly to clear the system from circulating cell-free DNA, foreign DNA from food digestion or potential pathogens, and endogenous chromosomal DNA from apoptotic and necrotic cells. Abnormal DNAse I activity occurs in association with a variety of cancers and auto-immune illnesses that exhibit elevated levels of cell-free DNA. Furthermore, DNAse I has been therapeutically used in cystic fibrosis patients to degrade DNA and reduce sputum viscosity. BioVision's DNAse I Activity Fluorometric Assay Kit 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.

DNA Probe —(DNAse I Sample / Inhibitor)→ Fluorescent DNA Product (Ex/Em= 651/681 nm)

II. Applications:

- Measurement of DNAse I activity of purified proteins
- Quantitative analysis of DNAse I mutants and inhibitors
- Comparative examination of DNAse I activity in biological samples

III. Sample Type:

- Purified Protein, serum.

IV. Kit Contents:

Components	K429-100	Cap Code	Part Number
10X DNAse I Assay Buffer	1.1 ml	Clear	K429-100-1
DNA Probe	1 vial	Blue	K429-100-2
DNA Probe Re-suspension Buffer	250 µl	Red	K429-100-3
DNAse I Positive Control	1 vial	Purple	K429-100-4
Positive Control Re-suspension Buffer	1 ml	Brown	K429-100-5
Molecular Biology Grade Water	25 ml	NM	K429-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom, low-medium binding
- Spectrophotometer
- Purified DNAse I, DNAse I inhibitors, biological samples

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Upon re-suspension, aliquot DNA Probe and DNAse I Positive control to avoid repeated freeze-thaw cycles. Read entire protocol before performing the assay.

- **10X DNAse I Assay Buffer:** Store at 4 ºC. Warm to 37 ºC temperature before use.
- **DNA Probe:** Reconstitute with 220 µl of DNA Probe Re-suspension Buffer. Aliquot and store at -20ºC. Avoid multiple freeze-thaw cycles.
- **DNA Probe Re-suspension Buffer:** Ready to use. Store at RT.
- **DNAse I Positive Control:** Reconstitute with 220 µl of Positive Control Re-suspension Buffer. Aliquot and store at -20°C.
- **Positive Control Re-suspension Buffer:** Ready to use. Store at -20°C.
- **Molecular Biology Grade Water:** Ready to use. Store at RT.

VII. DNAse I Activity Assay Protocol:

Caution! 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.

1. **Sample Preparation:** Thaw any purified enzymes and biological samples along with all the provided assay components on ice, unless otherwise stated. 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. Use water only (no enzyme/sample) for background control reaction. For positive control reaction, add 2 µl of DNAse I Positive Control to 48 µl of water. Mix well.

   Notes:

   a. Do not store enzyme/inhibitor/sample dilutions; discard the dilutions instead.

   b. The recommended amount of serum sample to use in the assay is 10-25 µl.

   c. For uncharacterized enzymes, we suggest testing several doses to ensure the reading is within the Standard Curve range.

d. If the user suspects any non-specific sample DNAse activity, 50 mM 2-Nitro-5-thiocyanatobenzoic acid (Cat. # B1316) can be used to specifically inhibit DNAse I activity.

2. **DNA Probe Standard Curve:** Prepare 1 µM DNA Probe stock by diluting 4 µl of 25 µM DNA Probe in 96 µl of molecular biology grade water. Add 0, 4, 8, 12, 16, 20 µl of 1 µM 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.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well containing sample(s), and standards prepare 50 µl Mix containing:

	Sample Reaction Mix (1 assay)	DNA Probe Standard Reaction Mix (1 assay)
10X DNAse I Assay Buffer	10 µl	10 µl
DNA Probe (25 µM)	2 µl	---
DNAse I Positive Control	---	2 µl
Molecular Biology Grade H_2O	38 µl	38 µl

Mix and 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.

4. **Measurement:** 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. Adjust GAIN/PMT setting of your fluorometer as necessary so that the standard curve readings are within the detection range of the instrument.

5. **Calculations:** *Standard Curve:* Record RFU at t = 90 min for each DNA Probe standard curve reading. Plot the DNA Probe standard curve with pmol of DNA on the x-axis and RFU on the y-axis. Apply a linear fit to the DNA standard values and determine the standard curve equation. *Samples/Positive Control:* Apply RFU values at each time point to the standard curve equation to determine pmol of DNA cleaved at each reaction time point. 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. Subtract background control readings from samples.

Sample DNAse I Activity = (slope/V) x D (pmol/min/ml ≡ µU/ml)

Sample Specific Activity = (slope/µg) x D (pmol/min/µg ≡ µU/µg)

Where: **V** = sample volume added into the reaction well (ml).
D = Dilution Factor
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.

a) **b)** **c)**

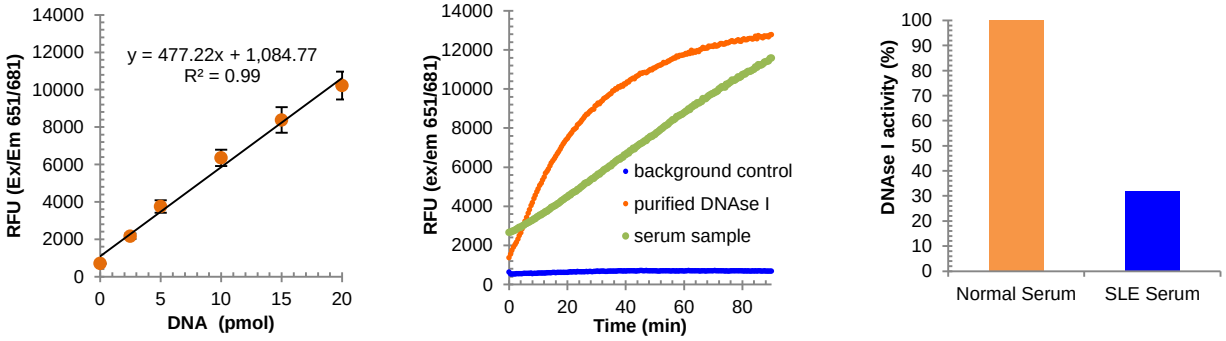

Figure: a) DNA Probe to Product conversion standard curve; b) representative activity curve for purified DNAse I (orange), serum sample (green), and background control (blue) at 37°C; c) comparative analysis of DNAse I activity from 25 µl undiluted single donor normal vs. Systematic Lupus Erythematosus (SLE) patient serum sample.

VIII. RELATED PRODUCTS

Apoptotic DNA Ladder Isolation Kit (K170)
Quick Apoptotic DNA Ladder Detection Kit (K120)
Enhanced Apoptotic DNA Ladder Detection Kit (K130)
2-Nitro-5-thiothiocyanatobenzoic acid (B1316)
DNase I Antibody (3214)

FOR RESEARCH USE ONLY! Not to be used on humans.