Technical Bulletin

DNAse I Activity Assay Kit (Fluorometric)

Catalog Number MAK397

Product Description

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 autoimmune 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.

The DNAse I Activity 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 λ_{Ex} = 651 nm/λ_{Em} = 681 nm. The limit of quantification is 178 fmoles of DNA probe cleaved per minute per mL.

The kit is suitable for the measurement of DNAse I activity of purified proteins, the quantitative analysis of DNAse I mutants and inhibitors, and the comparative examination of DNAse I activity in serum and other biological samples.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- 10X DNAse I Assay Buffer 1.1 mL
  Catalog Number MAK397A
- DNA Probe 1 vial
  Catalog Number MAK397B
- DNA Probe Resuspension Buffer 250 µL
  Catalog Number MAK397C
- DNAse I Positive Control 1 vial
  Catalog Number MAK397D
- Positive Control Resuspension Buffer 1 mL
  Catalog Number MAK397E
- Molecular Biology Grade Water 25 mL
  Catalog Number MAK397F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- DNAse-free pipette filter tips
- Fluorescence multiwell plate reader
- White flat-bottom low-medium binding 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 2-Nitro-5-thiocyanatobenzoic acid (Catalog Number N7009)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions.

Briefly centrifuge small vials prior to opening. Thaw the provided assay components on ice, unless otherwise stated.

10X DNAse I Assay Buffer: May be **s**tored at 4 °C. Warm to 37 °C prior to use.

DNA Probe: Reconstitute vial with 220 µL of DNA Probe Re-suspension Buffer. DNA Probe concentration upon reconstitution is 25 µM. Upon re-suspension, aliquot and store at -20 °C. Avoid multiple freeze-thaw cycles.

DNA Probe Re-suspension Buffer**:** Ready to use. Store at room temperature.

DNAse I Positive Control: Reconstitute vial with 220 µL of Positive Control Re-suspension Buffer. Upon re-suspension, aliquot and store at -20 °C. Avoid multiple freeze-thaw cycles.

Positive Control Re-suspension Buffer**:** Ready to use. Store at -20 °C.

Molecular Biology Grade Water**:** Ready to use. Store at room temperature.

Procedure

Caution! It is imperative to use Molecular Biology Grade Water for sample preparation and DNAse-free filter tips for sample pipetting at all times to avoid DNAse contamination.

Sample Preparation

1. Thaw purified enzymes and biological samples on ice.
2. Dilute enzymes, inhibitors, and biological samples to a desired concentration with Molecular Biology Grade Water or their corresponding storage buffer.

3. Add a desired amount of enzyme, inhibitor, or biological sample to each well and adjust the total volume to 50 µL with Molecular Biology Grade Water.
   a. For serum samples pipette 10-25 µL to appropriate wells of a 96-well plate.
   b. For uncharacterized enzymes, test several doses to ensure the reading is within the Standard Curve range.
4. Do not store enzyme/inhibitor/sample dilutions; discard the dilutions.
5. If non-specific sample DNAse activity is suspected, 50 mM 2-Nitro-5-thiocyanatobenzoic acid can be used to specifically inhibit DNAse I activity.

Background Control

Add 50 µL of Molecular Biology Grade Water to an appropriate plate well.

Positive Control

Add 2 µL of DNAse I Positive Control and 48 µL of Molecular Biology Grade Water to an appropriate plate well. Mix well.

Standard Curve Preparation

Prepare a 1 µM DNA Probe solution by diluting 4 µL of the 25 µM DNA Probe with 96 µL of Molecular Biology Grade Water. Prepare DNA Probe Standards in desired wells of a white flat-bottom 96-well plate according to Table 1. Mix well.

Table 1.
Preparation of DNA Probe Standards

Well	1 µM DNA Probe	Molecular Biology Grade Water	DNA Probe (pmol/ well)
1	0 µL	50 µL	0
2	4 µL	46 µL	4
3	8 µL	42 µL	8
4	12 µL	38 µL	12
5	16 µL	34 µL	16
6	20 µL	30 µL	20

Reaction Mix

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample, Positive Control, and Background Control, prepare 50 µL of Sample Reaction Mix according to Table 2, mix well.
   b. For each DNA Probe Standard well, prepare DNA Probe Standard Reaction Mix according to Table 2, mix well.

Table 2.
Preparation of Reaction Mixes

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

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

Measurement

Measure the fluorescence at λ_{Ex} = 651 nm/λ_{Em} = 681 nm in kinetic mode every 30 seconds for at least 90 minutes at 37 °C. Adjust GAIN/PMT setting of the fluorometer as necessary so that the standard curve readings are within the detection range of the instrument.

Results

Standard Curve:

1. Record RFU at T = 90 minutes 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.

Samples/Positive Control:

1. Subtract Background Control RFU 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.

Sample DNAse I Activity (pmol/min/mL or μU/mL) =

(Slope/V) x D

Sample Specific Activity (pmol/min/μg or μU/μg) =

(Slope/μg) x D

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 minute at 37 °C.

Figure 1.
Typical DNA Probe to Product conversion standard curve

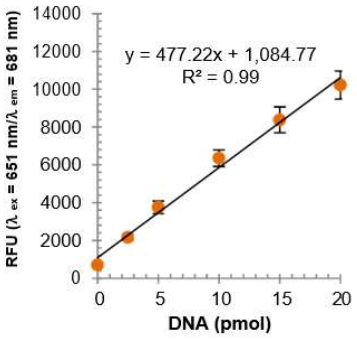


Figure 2.
Representative activity curve for purified DNAse I (orange), serum sample (green), and background control (blue) at 37 °C

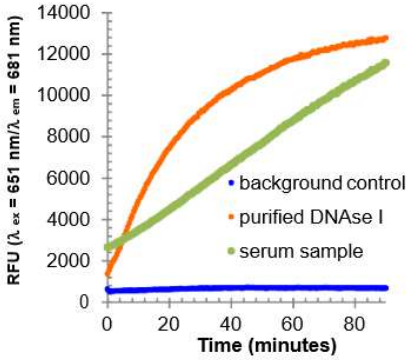
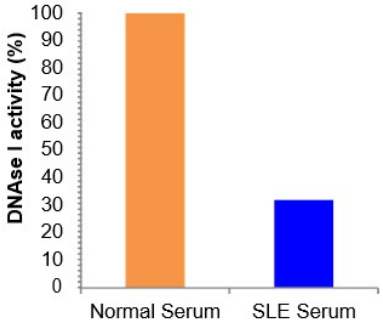


Figure 3.
Comparative analysis of DNAse I activity from 25 μL undiluted normal single donor serum vs. Systematic Lupus Erythematosus (SLE) patient serum sample.



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