Description

The 2-Step DNase I Assay Kit is a homogeneous fluorescent based assay kit designed to measure DNase I activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant DNase I, DNA substrate, GroovyGreen™ dye, assay buffer and co-factors for 100 enzyme reactions. The kit also contains EDTA as internal control.

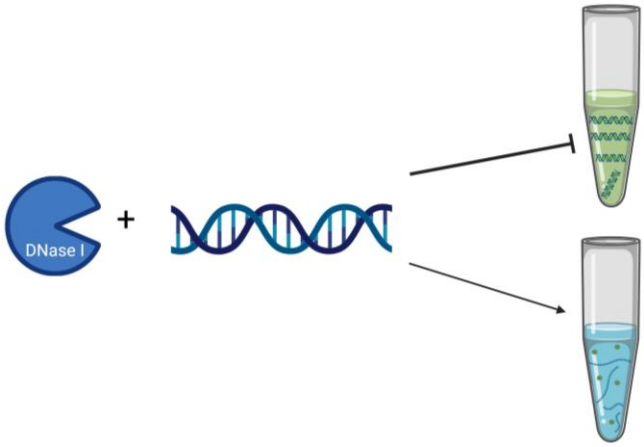

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Figure 1: 2-Step DNase I Assay Kit mechanism.

DNase I cleaves DNA molecules into nucleotides. The amount of nucleotide formed correlates directly with DNase I activity and can be quantified by the addition of a fluorescent dsDNA-binding dye which distinguishes dsDNA from free nucleotides. Since the dye emits fluorescence only when bound to the target dsDNA, the signal is inversely proportional to DNase I activity.

Background

DNase I, or deoxyribonuclease I, belongs to the DNase family of proteins. It is an endonuclease, and it can act on single- and double-stranded DNA (dsDNA), cleaving phosphodiester bonds located adjacent to pyrimidine nucleotides. DNase I is a commonly used tool in molecular biology, allowing degradation of DNA from RNA preparations, identification of protein binding sequences and preventing DNA related clumping in cell culture. It does not seem to have a preference for DNA bases, but it has higher sensitivity to minor grooves and the cleavage of purine-pyrimidine sequences. In the clinic, recombinant human DNase I has been used since 1993 in the treatment of patients with cystic fibrosis, by hydrolyzing the DNA present in the sputum of these patients. Its use in diseases where extracellular DNA is thought to play a role, such as chronic sinusitis and atelectasis, has also been evaluated. Interestingly, it may also have a potential application in SLE (systemic lupus erythematosus) by degrading DNA and preventing the formation of immune complexes. Deficiency in DNase can lead to anemia, cataracts and SLE. An understanding of its activity and inhibitors will provide useful insights into the mechanisms and therapies for extracellular DNA accumulation related diseases.

Applications

Study enzyme kinetics and screen small molecule inhibitors and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	DNase I (Bovine)*	100 U	-80°C
	Nicked DNA Substrate (20 ng/µl)	1 µg	-80°C
	10X DN-01 Assay Buffer 1 (Incomplete Buffer)	2 x 300 µl	-80°C
	0.5 M EDTA	50 µl	4°C
	200x GroovyGreen™ Dye	25 µl	-80°C
	0.5 M $MgCl_2$	30 µl	4°C
	1 M $MnCl_2$	15 µl	4°C
79685	96-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Fluorescence plate reader capable of measurement at λex502/λem523 nm.

Stability

This assay kit contains DNA and will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using EDTA as internal control if performing sticky DNA cutting assessment. If not running a dose response curve for the control inhibitor, we recommend running EDTA at 0.5X, 1X and 2X the IC_{50} value shown in the validation data below.
- The fluorescent signal intensity of GroovyGreen™ Dye may be affected by some salts and solvents. A signal intensity decrease is expected when $MnCl_2$ is used at the suggested concentration.
- If $MnCl_2$ is used, EDTA is not recommended as a control inactivator of the DNase I enzyme due to the presence of varying levels of free $MnCl_2$ (and therefore varied signal intensity decrease) in wells containing different concentrations of EDTA.

A. Blunt DNA Cut Studies

1. Prepare 1x Assay Buffer by diluting 10-fold the DN-01 Buffer 1 with distilled water.

2. Dilute 1M $MnCl_2$ 400-fold in 1x Assay Buffer to make Complete Assay Buffer.

3. Thaw DNase I on ice. Briefly spin the tubes to recover the full content.

4. Dilute DNase I to 0.01 units/µl in Complete Assay Buffer (10 µl/well).

5. Dilute Nicked DNA Substrate 20-fold in Complete Assay Buffer (10 µl/well).

6. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare 5-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   6.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in 1x Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   For positive and negative controls, prepare 5% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

7. Add 10 µl of diluted DNase I to all wells, except the "Negative Control" wells.

8. Add 10 µl of 1x Assay Buffer to the "Negative Control" wells.

9. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor".

10. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

    Note: Consider preincubating DNase I with the inhibitor for 30 minutes at Room Temperature (RT) prior adding the substrate.

11. Add 10 µl of diluted Nicked DNA Substrate to all wells.

12. Incubate the plate at 37°C for 30 minutes.

Component	Negative Control	Positive Control	Test Inhibitor
Diluted DNase I (0.01 units/µl)	-	10 µl	10 µl
1x Assay Buffer	10 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Nicked DNA Substrate (1 ng/µl)	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

13. Dilute GroovyGreen™ Dye 200-fold with 1x Assay Buffer (25 µl/well). This makes 1x GroovyGreen™ Dye.

14. Add 25 µl of 1X GroovyGreen™ Dye to all wells.

15. Read fluorescence intensity of the samples (λexcitation = 502/10 nm; λemission = 523/10 nm) in an appropriate microplate reader. Alternatively standard fluorescein wavelengths can be used (excitation ~480 nm, emission ~520 nm).

B. Sticky DNA Cut Studies

1. Prepare 1x Assay Buffer by diluting 10-fold the DN-01 Buffer 1 with distilled water.

2. Dilute 0.5 $MgCl_2$ 200-fold with 1x Assay Buffer. This makes Complete Assay Buffer.

3. Thaw DNase I, on ice. Briefly spin the tubes to recover the full content.

4. Dilute DNase I to 0.1 units/µl with Complete Assay Buffer (10 µl/well).

5. Dilute Nicked DNA Substrate 20-fold with Complete Assay Buffer (10 µl/well).

6. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare 5-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   6.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in 1x Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

For positive and negative controls, prepare 5% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

7. Add 10 µl of diluted DNase I to all wells, except the "Negative Control" wells.

8. Add 10 µl of 1x Assay Buffer to the "Negative Control" wells.

9. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor".

10. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

    Note: Consider preincubating DNase I with the inhibitor for 30 minutes at Room Temperature (RT) prior adding the substrate.

11. Add 10 µl of diluted Nicked DNA Substrate to all wells.

12. Incubate the plate at 37°C for 30 minutes.

Component	Negative Control	Positive Control	Test Inhibitor
Diluted DNase I (0.1 units/µl)	-	10 µl	10 µl
1x Assay Buffer	10 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Nicked DNA Substrate (1 ng/µl)	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

13. Dilute GroovyGreen™ Dye 200-fold with 1x Assay Buffer (25 µl/well). This makes 1x GroovyGreen™ Dye.

14. Add 25 µl of 1X GroovyGreen™ Dye to all wells.

15. Read fluorescence intensity of the samples (λexcitation = 502/10 nm; λemission = 523/10 nm) in an appropriate microplate reader. Alternatively standard fluorescein wavelengths can be used (excitation ~480 nm, emission ~520 nm).

Example Results

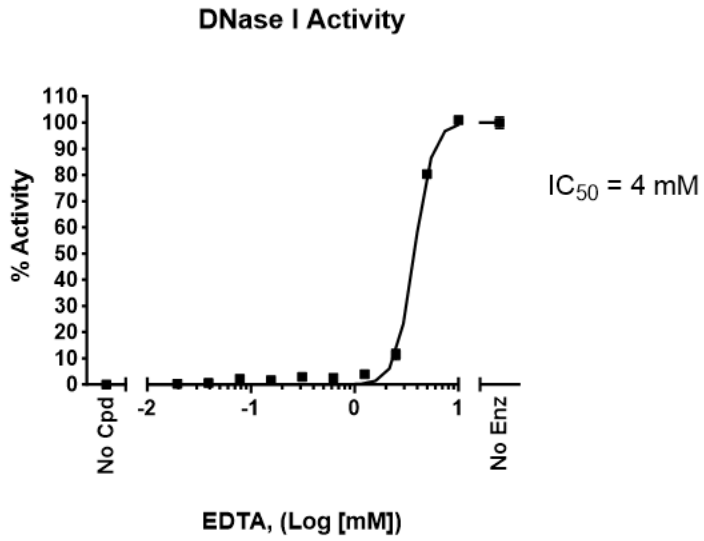

Figure 1. DNase I activity measured in the presence of EDTA.
DNase I activity was measured in $MgCl_2$ containing buffer and in the presence of increasing concentrations of EDTA.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Lazarus R. and Wagener J., 2019, *Pharmaceutical Biotechnology*: 471-488.

Related Products

Products	*Catalog #*	*Size*
DNA Polymerase γ Assay Kit	82098	96 reactions
DNA Polymerase β Assay Kit	82099	96 reactions
DNA Polymerase β (POLB), His-Tag Recombinant	21000	100 µg/1 mg
DNA Polymerase γ (POLG), His-FLAG-Tag Recombinant	21001	100 µg

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