Rev. 0

DNase Activity Assay-Fluorometric (DAA-F)

Catalog #8838
100 reactions

Product Description

DNase activity plays a critical role in DNA degradation, nucleic acid processing, and chromatin accessibility, making it essential for molecular biology research. In clinical studies, abnormal DNase activity is associated with diseases such as cystic fibrosis, autoimmune disorders, and cancer, where imbalances can serve as key biomarkers. Additionally, DNase enzymes are widely used in bioprocessing applications, including recombinant protein production and primary cell isolation.

The ScienCell DNase Activity Assay Kit (Fluorometric) provides a sensitive, quantitative method for detecting DNase I activity in purified enzyme samples, inhibitors, buffers/reagents and biological specimens. The assay measures enzymatic cleavage of a DNA probe, generating a fluorescent signal (Ex/Em: 480/520 nm) for precise activity monitoring. With high sensitivity and a quantification limit of 1.5 µU/mL, this kit is a reliable tool for DNase research across multiple applications.

Kit Components

Cat #	Component	Quantity	Storage
8838a	10X DNase Assay Buffer	1.1 mL	4°C
8838b	DNA Probe	1 vial	-20°C
8838c	DNase I Positive Control	1 vial	-20°C
8838d	Positive Control Resuspension Buffer	0.5 mL	RT
8838e	Nuclease-Free Water	15 mL	RT

Additional Materials Required (Materials Not Included in Kit)

96-well plate
Plate-reader
Purified DNase I, DNase I inhibitors, biological samples.

Quality Control

Standards with concentrations ranging from 4 to 20 pmole/well is used to generate a standard curve R^2 value >0.98.

Product Use

DAA-F is designed for rapid, sensitive quantification of DNase activity and inhibitors potency in solution-based samples. It provides high sensitivity, detecting DNase activity down to 1.5 µU/mL (pmol/min/mL), making it ideal for applications requiring precise enzyme activity measurements. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product ships on dry ice. Store the DNase I Positive Control (#8838c) and DNA Probe (#8838b) at -20°C in a manual defrost freezer, protected from light. Keep the 10X DNase Assay Buffer (#8838a) at 4°C and store the Positive Control Resuspension Buffer (#8838d) and Nuclease-Free Water (#8838e) at room temperature. Aliquot reagents before storage to minimize freeze-thaw cycles. Kit is stable for 6 months, if components are properly stored and have not been reconstituted.

Procedure

1. **Reagent Preparation**
   - 1.1. Reconstitute DNA probe (cat# 8838b) with 220 µL of Nuclease free water to make 25 µM DNA Probe stock. Aliquot and store at - 20ºC. Avoid multiple freeze-thaw cycles.
   - 1.2. Reconstitute DNase I Positive Control (cat# 8838c) with 500 µL of Positive Control Resuspension Buffer to make 50X DNase Positive Control. Aliquot and store at -20°C.
     Note: Prepare the reagent on the day of the experiment, and ensure that the reagents have reached room temperature.

2. **Preparation of the DNA Standard Curve**
   - 2.1. Prepare a fresh set of standards for each assay. Discard any remaining working dilutions after use, as they are not stable for storage
   - 2.2. To prepare a 1 µM DNA Probe stock, dilute 6.2 µL of 25 µM DNA Probe in 148.8 µL of Nuclease free water (cat# 8838e).
   - 2.3. Follow Table 1 to prepare the standard dilution series.
   - 2.4. Mix each standard thoroughly and transfer 50 µL per well in duplicate to ensure accuracy.

Table 1.

DNA probe concentration (pmole/well)	0	4	8	12	16	20
1 µM DNA Probe (µL)	0	10	20	30	40	50
Nuclease Free Water (µL)	125	115	105	95	85	75

3. **Sample Preparation**
   - 3.1. Dilute biological samples, enzymes, or inhibitors to the desired concentration using Nuclease free water or their respective storage buffer.
   - 3.2. Transfer the appropriate volume of each sample into the designated wells, and adjust the volume to 50 µL per well.
     Note: For samples with unknown range of enzymatic activity, we recommend to test multiple concentrations to ensure the reading is within the standard curve range.

4. **Assay Procedure**
   - 4.1. For background control, use Nuclease free water only.
   - 4.2. To prepare a 1X DNase Positive Control, dilute the 50X reconstituted DNase with nuclease-free water. For example, mix 2 µL of 50X DNase I Positive Control with 98 µL of nuclease-free water. Ensure thorough mixing before use.
   - 4.3. For the positive control, add 2 µL of 1X DNase I positive control to 48 µL of Nuclease free water.

4.4. For inhibitor testing, add 2 µL of 1X DNase I positive control, followed by the inhibitors. Adjust the volume to 50 µL per well using nuclease-free water.
4.5. Prepare the sample reaction mix by combining:
- 10 µL of 10X DNAse Assay Buffer (cat# 8838a)
- 2 µL of DNA probe (25 µM)
- 38 µL of Nuclease-Free Water

4.6. Prepare the standard reaction mix by combining:
- 10 µL of 10X DNase Assay Buffer
- 2 µL of 1X DNase positive control
- 38 µL of Nuclease Free Water

4.7. Add 50 µL of the sample reaction mix to each sample well, including background and positive controls.
4.8. Add 50 µL of the standard reaction mix to wells designated for the DNA probe standard.
4.9. Measure fluorescence at excitation 480 nm and emission 520 nm in kinetic mode, recording readings every 1 minute for 10–60 minutes, or until the lowest concentration produces at least four consecutive linear data points.

5. **Data Analysis:**
5.1. Subtract background control RFU values from standards and sample readings.
5.2. Generate a standard curve by plotting DNA probe concentration (pmol) on the x-axis against RFU values on the y-axis.
5.3. Verify the linearity and correlation coefficient of the standard curve.
5.4. Use the standard curve equation to determine the pmol of DNA cleaved at each reaction time point.
5.5. Plot pmol of cleaved DNA (y-axis) against reaction time in minutes (x-axis) and calculate the reaction rate (pmol/min) from the linear portion of the curve.
5.6. Use the equation below to calculate DNase activity as pmol of DNA cleaved per minute per mL of sample, which is also expressed in microunits per mL (µU/mL).

$$\textbf{Sample DNase Activity} = \frac{Slope}{V} \times D = \frac{pmole}{min.\ mL} = \mu U/mL$$

5.7. Use the equation below to calculate DNase activity per microgram of protein, which is useful for comparing enzyme activity independent of concentration.

$$\textbf{Sample Specific Activity} = \frac{Slope}{\mu g} \times D = \frac{pmole}{min.\ \mu g} = \ \mu U/\mu g$$

Where:
V = sample volume added in the sample wells (mL).
D = sample dilution factor if the sample was diluted before testing**.**
Slope = The linear portion of the reaction curve, representing the DNA cleavage rate in pmol/min.

Unit Definition: One unit (U) of DNase I activity is defined as the amount of enzyme required to cleave 1 µmol of DNA per minute under the assay conditions.

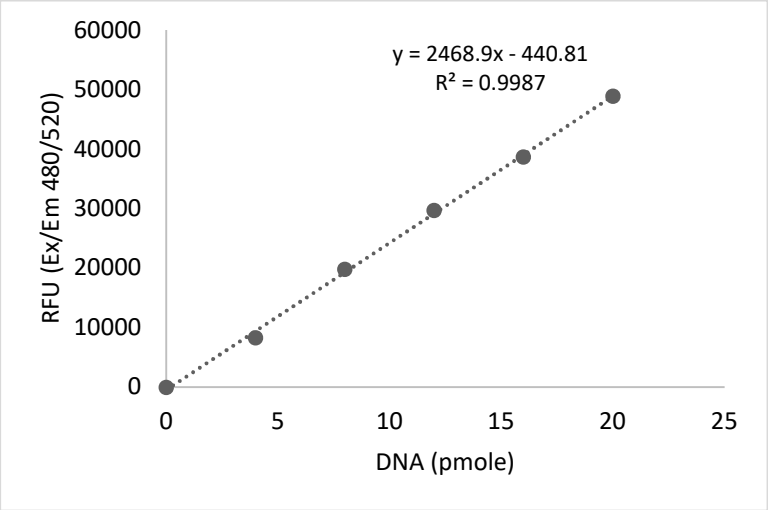

Figure 1. A typical of a DNA probe standard measured with ScienCell DNase Activity Assay.

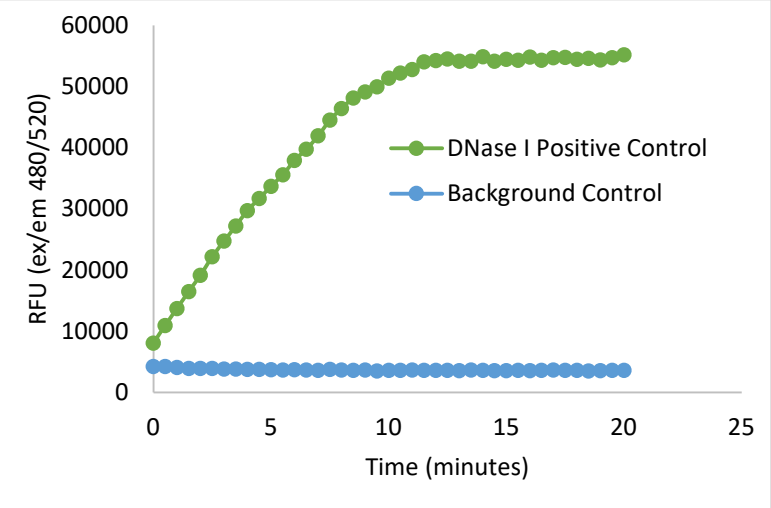

Figure 2. Representative activity curve for 1X DNase I Positive Control (green) and background control (blue).