resDetect™ DNase Activity Assay Kit (Fluorescence)

Cat. No. ASE-A002 **Size : 96 tests / 480 tests**

Background

Deoxyribonucleases (DNases) are enzymes which are able to hydrolyze phosphodiester bonds of DNA molecules. They can be divided into two families, which differ in biochemical and biological properties—DNase I and DNase II families. The presence of DNases would affect many experimental results like PCR, so it is necessary to evaluate the presence of DNases.

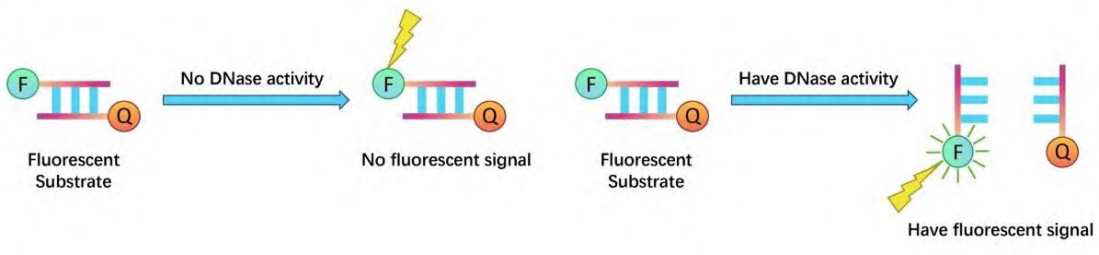
DNases are ubiquitous in both the environment and many biological materials. Many molecular biology experiments rely on the use of plastics, chemicals, and solutions that are free from detectable DNase activity, once these materials are contaminated with DNases, the experiments will be affected because of the DNases can degrade DNA. Since even only minute amounts of DNase contamination would ruin the experiment, it is necessary to evaluate the presence of DNase with a reasonable method.

Published methods for detecting DNase such as Nucleic acid hydrolyzed gel electrophoresis and ultraviolet spectrophotometer are typically time consuming, not quantitative, and relatively insensitive. The other methods such as HPLC and electrochemical methods are limited to instrumentation.

In contrast, the DNase Activity Assay Kit (Fluorescence) can detect DNase contamination within 30 minutes, and the kit is high sensitivity, easy to use. Moreover, the DNase and RNase Activity Assay Kit (Fluorescence) have been designed to work together seamlessly for simultaneous quantitative detection of DNases and RNases in a single sample.

Detection Principle

The DNase Activity Assay Kit (Fluorescence) is based on a fluorophore-labeled DNase substrate. When the sample does not contain DNase activity, the substrate is stable and does not produce a fluorescent signal; when the sample contains DNase activity, the substrate is degraded, resulting in a gradual enhanced fluorescence signal, the rate of increase in fluorescence signal is positively correlated with the number and activity of enzymes. Use a fluorescence microplate reader to measure at the wavelength of ex/em = 535/565 nm to determine whether the sample is contaminated by DNase.



Applications

The DNase Activity Assay Kit (Fluorescence) is a convenient and sensitive assay tool to test the presence of DNase in buffers, reagents, and other components.

Kit Composition

ID	Items	96 Tests	480 Tests	Storage
ASE2-C01	DNase Substrate	2 nmol	10 nmol	-20°C, avoid light
ASE2-C02	10X Reaction Buffer for DNase	10 mL	10 mL	-20°C
ASE2-C03	DNase I (1 U/μL)	20 μL	50 μL	-20°C
ASE2-C04	TE Buffer (pH 8.0)	1.5 mL	6 mL	-20°C
ASE2-C05	Nuclease-free Water	10 mL	50 mL	-20°C

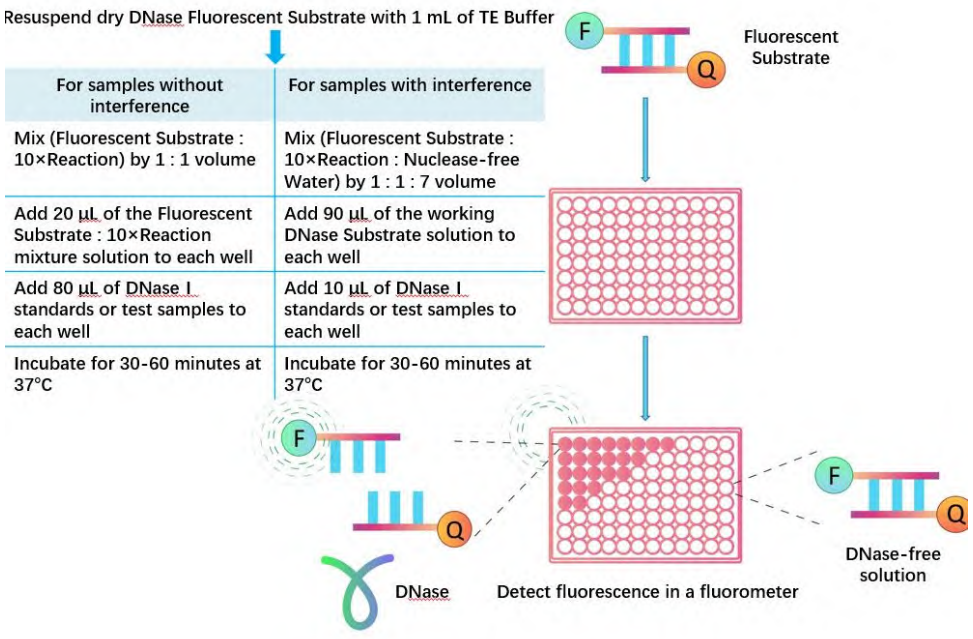
Materials required but not provided

Items	Specifications	Source
Nuclease-free pipettors, and tips	Nuclease-free	For example, pipettors, and tips from RAININ
Nuclease-free black 96-well plates	Nuclease-free, black non-transparent 96-well plates typically give the lowest background signal	For example, Corning 96 Well Black plates (Cat. No. 3924)
Nuclease-free EP tube	Nuclease-free	-
96-well fluorescence plate reader	Plate reader capable of measuring two or more fluorescent wavelengths in kinetic mode	For example, BMG CLARIOstar Plus Multi-Mode Microplate Reader

Shipping and Storage

1. The product is shipped at dry ice conditions.
2. The unopened kit is stable for 12 months from the date of manufacture if stored at -20℃.
3. The opened kit is stable for up to 3 months from the date of opening at -20℃.
4. If the reconstructed DNase Substrate Solution can't be used out at once, store it at -25~-15°C to avoid repeated freezing and thawing more than 3 times.
5. Do not use reagents past their expiration date.

Quickguide



Preparation before experiment

1. Experimental environment preparation: In order to ensure the accuracy of the experiment, the experimental environment requires that the operation process does not introduce additional DNase. Before you begin, the laboratory can be ultraviolet disinfection for 30 minutes, the experimental operation is carried out in an ultra-clean bench, clean the operation surface of the ultra-clean bench, and open the ultra-clean bench for ultraviolet irradiation for above 30 minutes.

2. Clean equipment surface, if a fluorometer will be used, turn it on and set the following parameters:

Mode	**Kinetic mode using a 96-well plate (if available)**
Excitation/emission (ex/em) maxima	535/565nm
Gain	Set the gain to autoscale if possible. Alternatively use a medium gain setting initially. Note: the setting method of different instruments is not consistent, please consult the instrument supplier for details.
Data collection	Intermittent, 1–1.5 min increments. Use intermittent data collection to limit photobleaching.
Temperature	37°C

3. Materials Preparation: Prepare materials and tools for your experiment, such as Nuclease-free pipettors, and tips, black 96-well plates, EP tubes, the details could refer to "***Materials required but not provided***" in page 2.

4. Reagent Preparation: Take out the kit, equilibrate all the buffer components and DNase I standard to room temperature, make sure that all solution (10×Reaction Buffer, TE Buffer, Nuclease-free Water and DNase I standard) are fully thawed and evenly mixed.

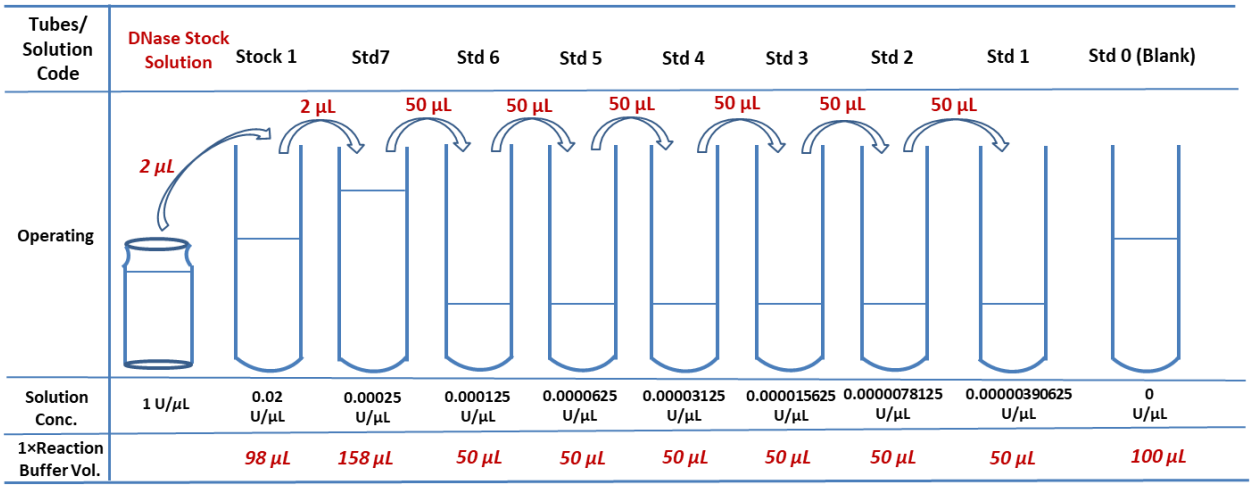
5. DNase Substrate Solution (2 nmol/mL) Preparation: Resuspend 1 tube of lyophilized DNase Substrate with 1 mL of the supplied TE Buffer (pH 8.0), leave it on the ice box for 30 minutes, dissolve the DNA Substrate completely. If the DNase Substrate Solution can't be used out at once, store it at -25~-15°C to avoid repeated freezing and thawing more than 3 times.

Procedure for assay

1. **Prepare Dilution Buffer (1×Reaction Buffer)** by diluting the 10×Reaction Buffer with Nuclease-free Water. Calculate the required 1×Reaction Buffer volume, for example, when 1 mL of 1×Reaction Buffer is required, add 0.1 mL of 10×Reaction Buffer into 0.9 mL of Nuclease-free Water.
2. **Prepare the samples**
   All samples with a concentration above the highest standard (Std 7) must be diluted in 1×Reaction Buffer.
   Different type of samples can be test by the kit, such as some biological materials, buffers, and solid surfaces. Because nuclease activity is greatly affected by pH and salt, you should know the exact composition of your samples and solution incompatibility, some samples may need to be diluted to reduce interference, the specific requirements for samples and solutions could refer to "***Frequently asked questions (FAQs)***" in page 9.
   If your sample is a lyophilized powder, and needs to be reconstructed, please reconstitute the sample following the COA, and note that any reconstituted solution should be Nuclease-free.
3. **Prepare the working DNase I standards**
   Each well requires 10 µL or 80 µL of standard according to the method. Serially dilute the **DNase I** standard stock solution with the Dilution Buffer used to prepare your standards. To avoid introducing extra DNase I, all tips and EP tubes and other consumables should be Nuclease-free, and all buffers also should be Nuclease-free. In order to counteract any standard sticking, we recommend changing tips between each dilution.

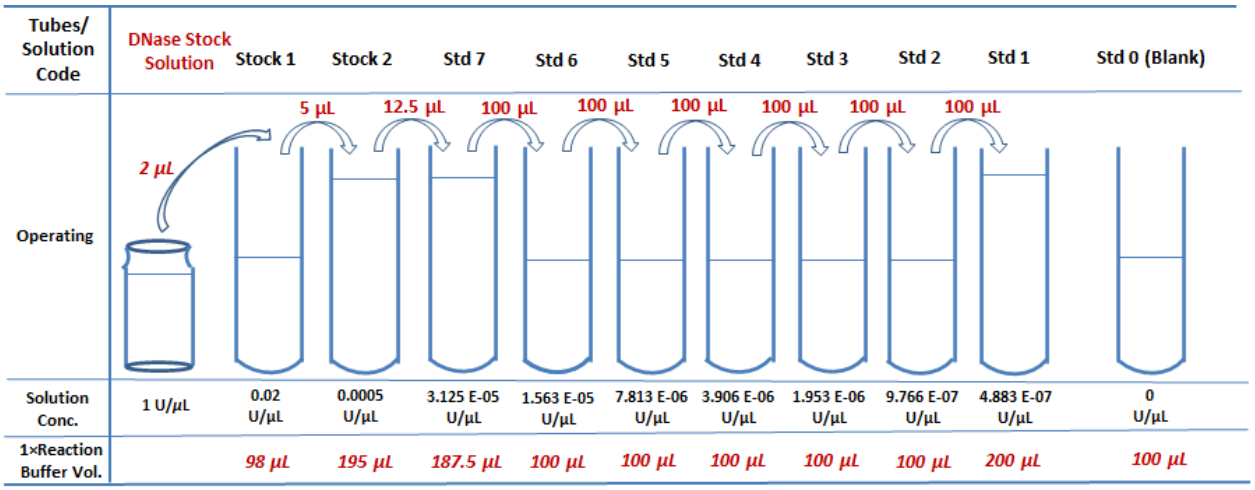
When the required sample volume is 10 µL, a recommended DNase I standard dilution procedure is listed and illustrated below (1×Reaction Buffer is recommended as the Sample Dilution Buffer):

The concentration of the reconstituted DNase I standard stock solution is 1 U/µL, prepare Stock1 by diluting 2 µL DNase I stock solution into 98 µL Sample Dilution Buffer, mix gently well. Then prepare the highest concentration of standard curve, Std 7 (0.00025 U/µL), by diluting 2 µL Stock1 into 158 µL Sample Dilution Buffer. Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 50 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



When the required sample volume is 80 µL, a recommended DNase I standard dilution procedure is listed and illustrated below (1×Reaction Buffer is recommended as the Sample Dilution Buffer):

The concentration of the reconstituted DNase I standard stock solution is 1 U/µL, prepare Stock1 by diluting 2 µL DNase I stock solution into 98 µL Sample Dilution Buffer, mix gently well. Then prepare Stock2 by diluting 5 µL Stock1 into 195 µL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std 7 (3.125E-05 U/µL), by diluting 12.5 µL Stock2 into 187.5 µL Sample Dilution Buffer. Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 100 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



4. Prepare the DNase Substrate working solution

When the required sample volume is 80 μL, each well requires 10 μL of DNase Substrate stock solution and 10 μL of 10×Reaction Buffer, mix the DNase Substrate and 10×Reaction Buffer by 1:1 volume, and then add 20 μL of the mixture solution to each well.

When the required sample volume is 10 μL, each well requires 90 μL of DNase Substrate working solution. Calculate the required total volume of DNase Substrate working solution according to the wells number in the experiment. Add equal volume of 2 nmol/mL DNase Substrate and 10×Reaction Buffer into 7 times the volume of Nuclease-free Water. For example, When the number of experimental wells is 20, 1.8 mL of DNase Substrate working solution is required, we can prepare 1.89 mL of DNase Substrate working solution to ensure a margin, add 210 μL of 2 nmol/mL DNase Substrate and 210 μL of 10×Reaction Buffer into 1.47 mL of Nuclease-free Water, up to 1.89 mL of the working DNase Substrate solution to be tested.

Please refer to the following methods to prepare the solution:

Tests	Working solution	DNase Substrate	10×Reaction Buffer	Nuclease-free Water
8 Tests	810 μL	90 μL	90 μL	630 μL
20 Tests	1890 μL	210 μL	210 μL	1470 μL

5. Add the above prepared samples, substrate, and DNase I standards to the plate wells as required, and read the fluorescence signal value.

Standards and samples can be added as shown below:

It is recommended that each concentration of standards and your samples be reperforated. If you need to add your own positive references and negative references, the number of wells for the positive references should be not less than 1, and the number of wells for the negative references should be not less than 2:

1) If you have access to a fluorometer capable of collecting data in real-time:

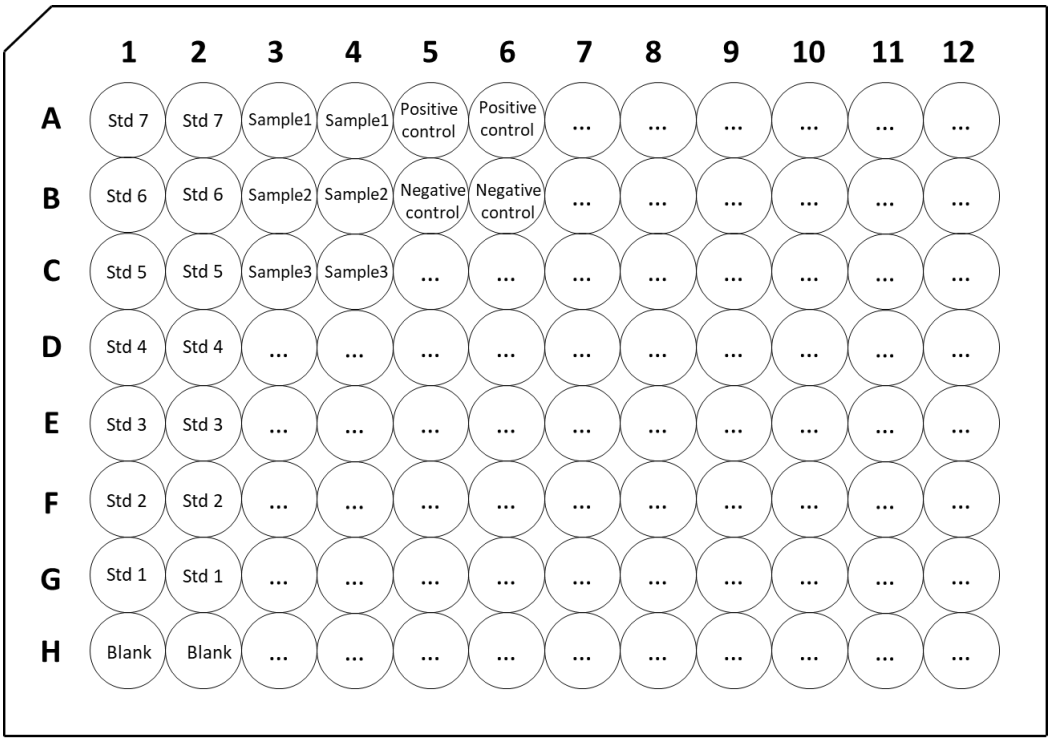
Add the corresponding volume of working DNase Substrate solution, DNase I standards or samples to each 96-well plate, incubate the plate in the fluorometer collecting real-time data at 1~1.5 minutes intervals for 30~60 minutes at 37°C using the settings described in this section. The DNase Activity Assay can be evaluated in rigorous kinetic terms. Using real-time data, DNase activities can be compared using enzyme velocity measurements.

2) If you do not need real-time data from the DNase Activity Assay, you can measure the fluorescence signals by endpoint measurement using a fluorometer.

Add the corresponding volume of working DNase Substrate solution, DNase I standards or samples to each 96-well plate, after incubate for 30~60 minutes at 37°C, measure the sample fluorescence using the settings described in this section.

Note: The DNase standards and all test samples should be measured at the same gain setting in the same plate.

Plate Layout



Data processing & Interpretation

Sample Type	Expected Result
DNase Standards	All 7 DNase Standards were positive, take RFU (std 0) and RFU (standard) as the ordinate and DNase concentration of standard as the abscissa, fitting the standard curve with four parameters logistic model, and the correlation coefficient R^2 should be ≥ 0.99.
Positive References	The signals of Positive references should be positive, and the concentration calculated back according to the standard curve should conform to the nominal concentration.
Negative references	The negative references have a minimal fluorescence (background). And the value is basically close to blank control value.
Test samples	If RFU of sample $\geq 2\times$RFU of blank, it is considered that the sample to be tested is contaminated by DNase. If the sample contains interfering substances, it may result in false negative results, at this time, the sample to be tested shall be pre diluted with nuclease-free water, and then tested.
Blank	The signals of blank (std 0) is the background value of the experiment should be as low as possible, the background value is different because of different fluorometer. All other samples are judged against this value.

Typical Data

Real-time fluorescent monitoring of DNase I activity in DNase Activity Assay Kit:

Add 90 µL of the working DNase Substrate solution to each 96-well plate, and add 10 µL of DNase I standards (0.00000390625-0.00025 U/µL×10µL/well = 0.0000390625-0.0025U/well), incubate the plate in the fluorometer **(BMG CLARIOstar)** collecting real-time data at one minute intervals for 30 minutes at 37°C using the settings described in this section. The DNase Activity Assay can be evaluated in rigorous kinetic terms using real-time data.

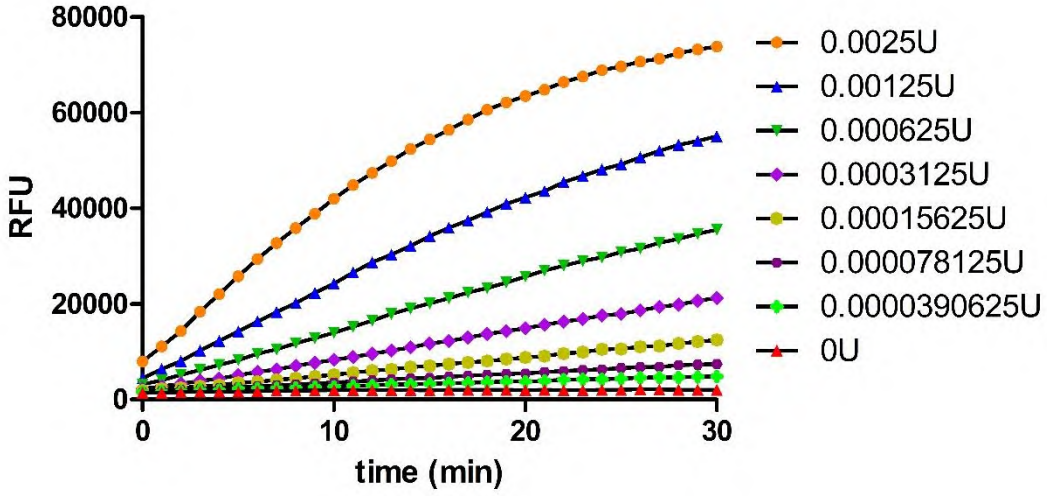
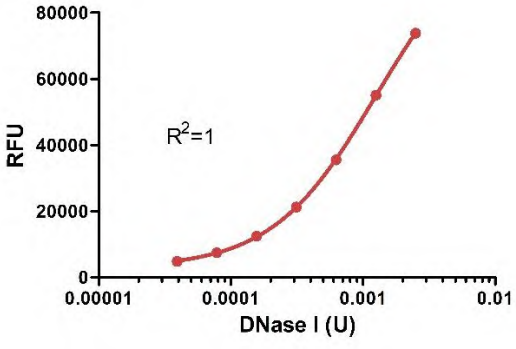


Figure1 Real-time fluorescent monitoring of DNase I activity

Standard curve of DNase Activity Assay Kit (Fluorescence):

This assay kit employs a standard detection of DNase I. Add 90 µL working DNase Substrate solution to each 96-well plate, and add 10 µL DNase I standards (0.00000390625-0.00025 U/µL×10 µL/well = 0.0000390625-0.0025 U/well), incubate for 30 minutes at 37°C. Then measure the fluorescence using the settings described in this section in a fluorometer **(BMG CLARIOstar)**. Take RFU of standards as the ordinate and DNase concentration as the abscissa. Four parameters logistic are used to draw the standard curve. This following data is for reference only.



(10 µL/well)	RFU-1	RFU-2	Ave (RFU)
0 U	2064	1971	2018
0.0000390625 U	4529	4517	4523
0.000078125 U	7009	6792	6901
0.00015625 U	11610	11201	11406
0.0003125 U	20195	19281	19738
0.000625 U	34274	33272	33773
0.00125 U	54299	51571	52935
0.0025 U	73625	71905	72765

Figure2 Standard curve of DNase I activity

Sensitivity

Assay range (U/μL/U/well)	Limit of quantification (LoQ*)
0.00000390625-0.00025 U/μL×10μL/well = 0.0000390625-0.0025U/well	0.0000390625 U
4.883 E-07 -3.125 E-05 U/μL×80μL/well = 0.0000390625-0.0025U/well	0.0000390625 U

Intra-Assay Statistics

Sample	1	2	3	4	5	6	7
Number of Replicate	8	8	8	8	8	8	8
Mean RFU	2703	4170	6804	11847	19904	32163	45024
Standard Deviation	30	87	182	340	538	455	713
Coefficient of Variation (%)	1.1	2.1	2.7	2.9	2.7	1.4	1.6

Inter-Assay Statistics

Sample	1	2	3	4	5	6	7
Number of Replicate	8	8	8	8	8	8	8
Mean RFU	2987	4711	8021	13914	23835	37728	51370
Standard Deviation	291	504	1025	1879	3323	5016	6482
Coefficient of Variation (%)	9.8	10.7	12.8	13.5	13.9	13.3	12.6

Recovery

	System	55 μg/mL of Pyrophosphatase (n=2)		20 μg/mL Thermostable Inorganic Pyrophosphatase (n=2)		10% of (50mM Tris-HCl and 50% Glycerol) (n=2)		1×Reaction Buffer (n=2)	
Sample	Enzyme activity. (U)	Calculated enzyme activity. (U)	Ave % RE	Calculated enzyme activity. (U)	Ave % RE	Calculated enzyme activity. (U)	Ave % RE	Calculated enzyme activity. (U)	Ave % RE
Sample 1	0.002	0.002132	107	0.00201	100	0.002013	101	0.002183	109
Sample 2	0.0005	0.000534	107	0.000491	98	0.000502	100	0.000565	113
Sample 3	0.0001	0.000110	110	0.000101	101	0.000105	105	0.000118	118

Frequently asked questions (FAQs)

1. Which enzymes can this kit detect except for DNase I?

In addition to DNase I, the DNase Activity Assay Kit (Fluorescence) is also optimized for the detection of Exonuclease III, Bal31 nuclease, micrococcal nuclease, Benzonase™ nuclease, mung bean nuclease, S1 nuclease, and T7 endonuclease.

2. Which solutions are incompatible?

Most reaction buffers and solutions that are used with DNA can be tested with DNase Activity Assay Kit (Fluorescence). Below are listed the types of solutions that are not compatible with kit:

Incompatible solutions	Notes
Gel loading buffers and other darkly colored solutions	Darkly colored solutions may interfere with excitation of the fluorophore or may block its light emission, making them incompatible with DNase Activity Assay Kit (Fluorescence).
Solutions that inhibit DNase activity	The following solutions are known to inhibit DNase: • Solutions with high ionic strength (e.g. 5 M NaCl, 20X SSC, 3 M sodium acetate, etc.) • Solutions with pH <4 or >9 • Chaotropic agents, detergents, chelating agents, or any solutions that denature proteins (e.g. SDS, guanidine thiocyanate, urea, EDTA, etc.)
Solutions that cause chemical instability of the DNase Activity Assay Kit (Fluorescence)	Solutions that chemically degrade the substrate may produce false positive signals. The DNase Substrate is unstable in the following types of solutions: • Solutions with pH >9 • Caustic solutions (strong acids and bases, bleach)

3. How to determine solution compatibility?

1) Test the solution following the standard procedure.
2) At the end of the incubation, if no fluorescence above the minus-DNase control is seen, add 5 µL of the supplied DNase I to the completed reaction, and repeat the incubation and signal detection. Compatible solutions will strongly fluoresce after incubation with DNase I.

4. How to test solid surfaces?

Pipette tips, pH electrodes, glass beads and other solid surfaces can be tested for DNase by preparing a mock DNase reaction as described for the minus-DNase control. Immerse the object in the reaction mixture for a few minutes (pipet up and down for pipette tips), and then check the solution for fluorescence as described in the protocol.

5. What should be done when suspected false positive or false negative results appearing?

1) In case of false positive in the experiment, firstly, the experimental consumables are excluded from nuclease contamination. Secondly, the experimental solution is inspected to ensure that all solutions are free of nuclease contamination and have no degradation effect on the DNase Substrate. At last, make sure there is no additional nuclease introduced by error operations.
2) When the experiment has false negative results, check whether the solution has DNase activity inhibitor or fluorophore blocking component. An inappropriate solution will produce false negative results.