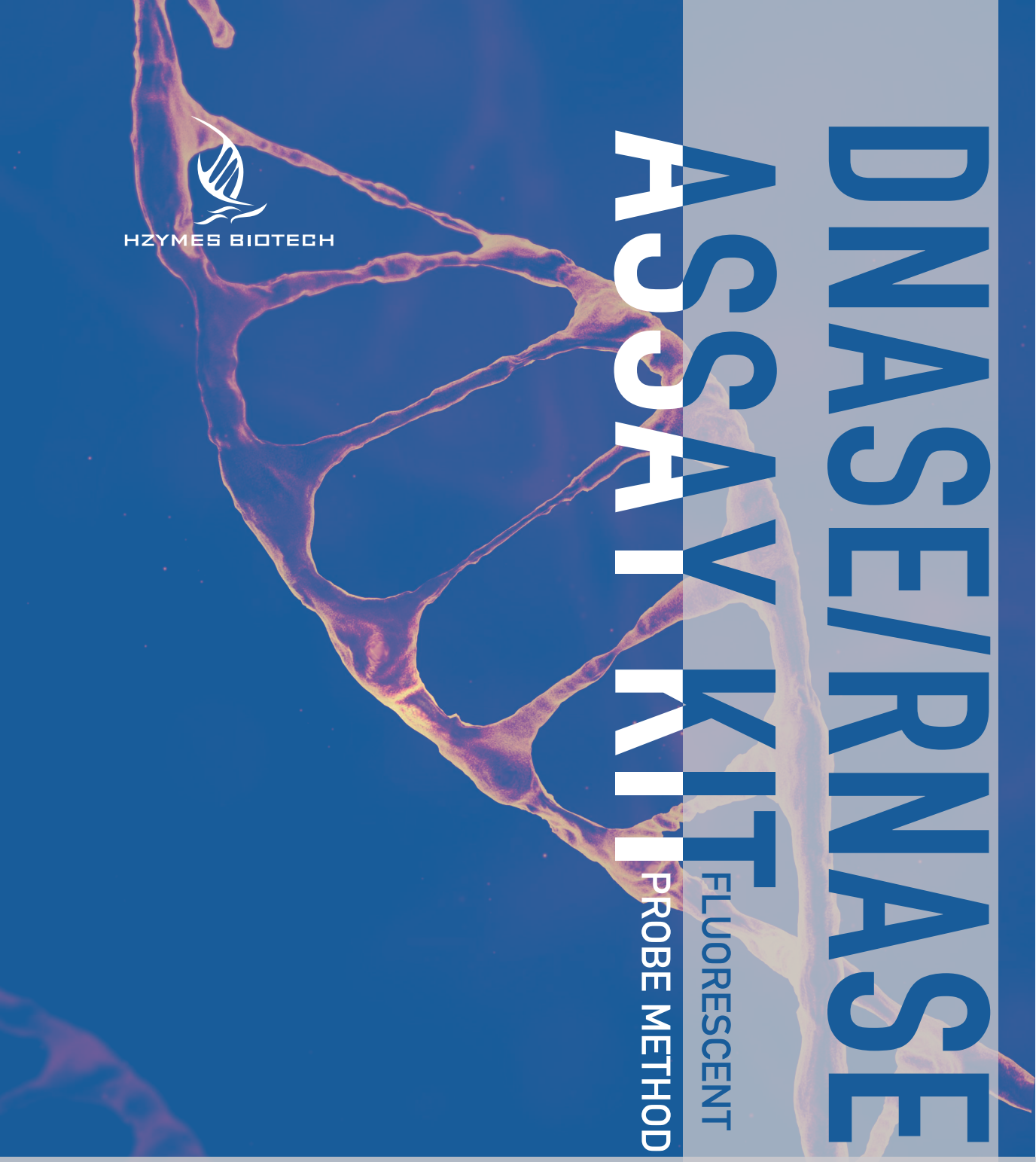
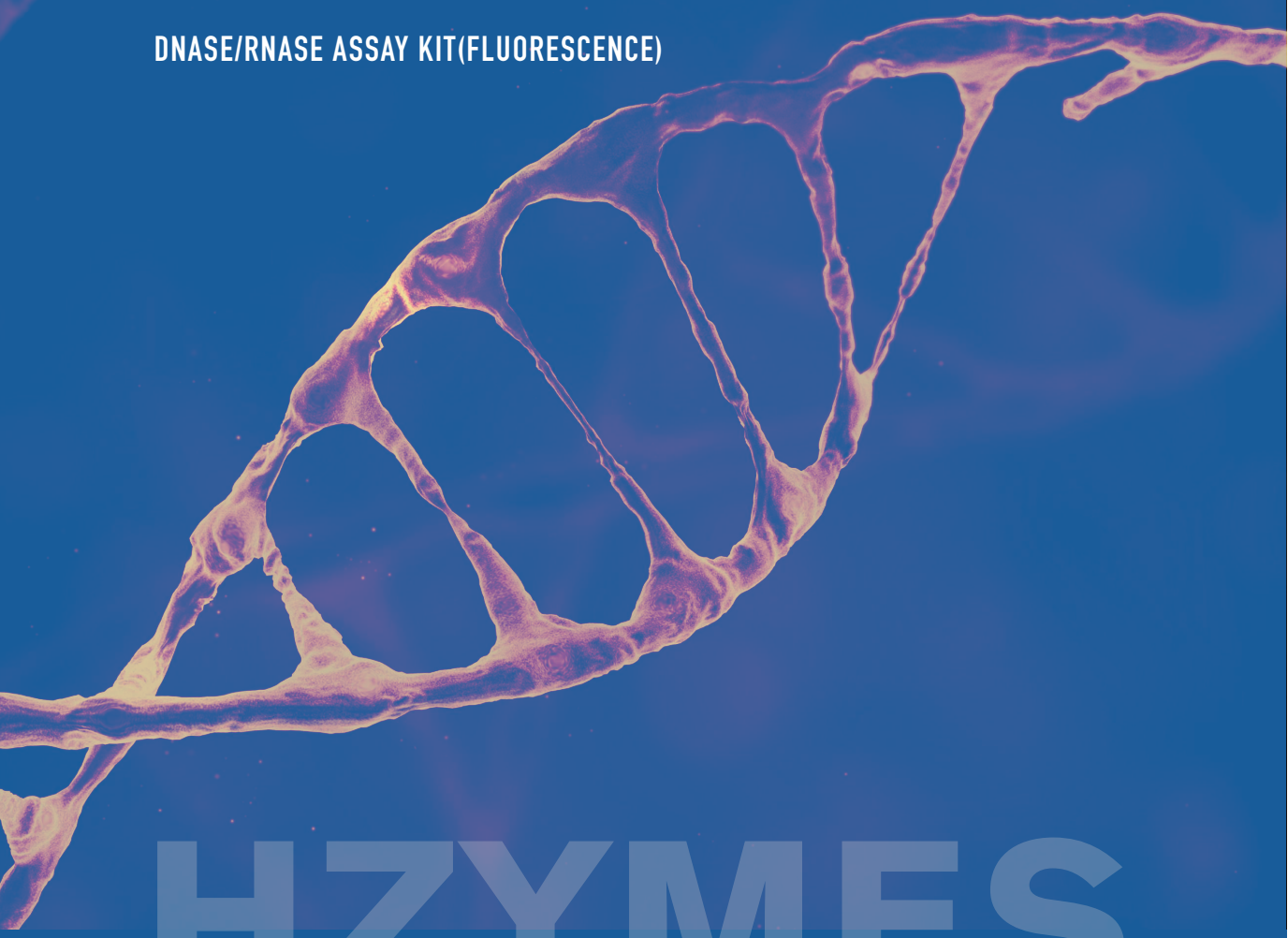


DNASE/RNASE ASSAY KIT(FLUORESCENCE)

HZYMES BIOTECHNOLOGY CO.,LTD

DNASE/RNASE ASSAY KIT(FLUORESCENCE)



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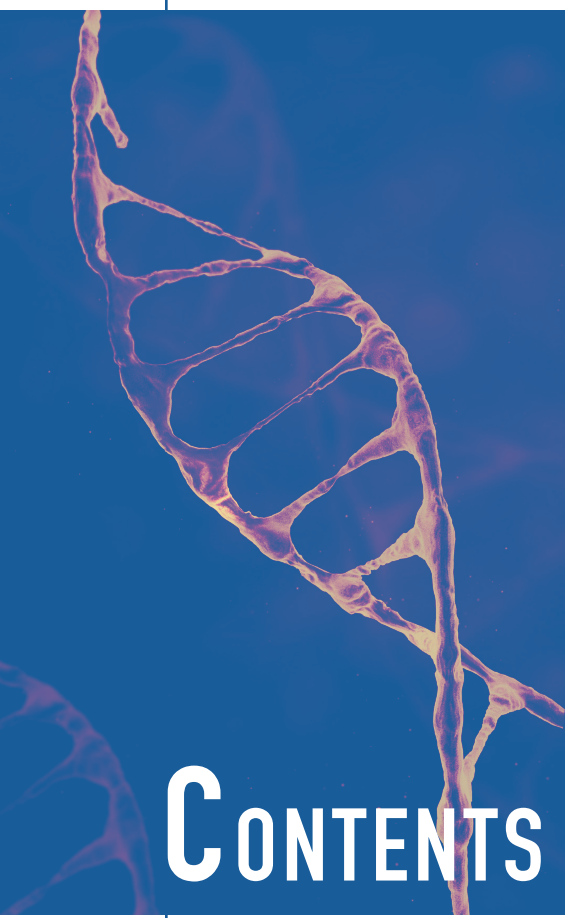


CONTENTS

DNase / RNase Assay Kit(Fluorescence) P01

Background
Detection Method
Pictures

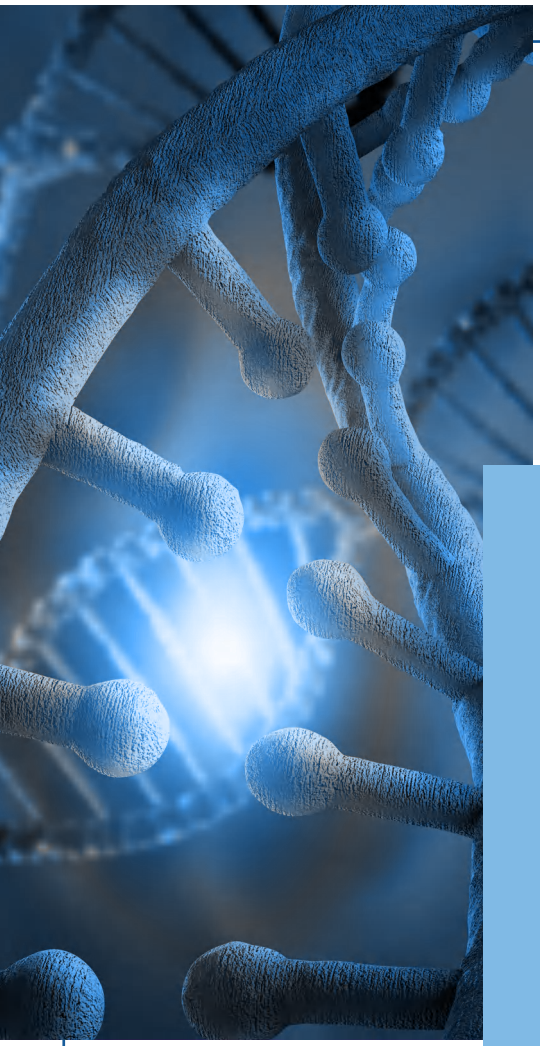
DNase Assay Kit(Fluorescence) P03

Application
Characteristics of products
Sensitivity higher than gel electrophoresis method
Sensitivity higher than competitor’ s
Kit composition
Equipment and consumables required
Preparation before experiment(check the user guide for more details)
Q&A
Product details

RNase Assay Kit(Fluorescence) P09

Application
Characteristics of products
Sensitivity higher than gel electrophoresis method
Sensitivity higher than competitor’ s
Kit composition
Equipment and consumables required
Preparation before experiment(check the user guide for more details)
Q&A
Product details

Background



In recent years, the application of mRNA synthesis technology has become more and more extensive, and the requirements for quality control in the process of mRNA vaccine and drug research and production are also increasing. Deoxyribonucleases (DNases) and ribonucleases (RNases) are ubiquitous enzymes that are present in both the environment and many biological materials. They can degrade DNA or RNA, affecting many research and production processes involving DNA and RNA. Due to the fragility of mRNA molecules, it is extremely important to inhibit or eliminate nuclease activity in research and production environments.

To this end, we need a fast and sensitive method to detect nucleases in the environment (such as laboratory benches, pipette tips, storage bags, reactors and other consumables)and materials (such as antibodies, enzymes, buffers), etc. activity, determine the presence of DNases or RNases contamination and quantitatively assess the extent of contamination.

For defining pollution-free methods, there are no clear regulations in the "USP43","EP10","Chinese Pharmacopoeia 2020 Edition" and other documents. The limit of quantification of nucleic acid hydrolysis-UV spectrophotometry is only 0.01U/μL, which is not suitable for the detection of micro nuclease’ s activity.

The nucleic acid hydrolysis-gel electrophoresis method is greatly affected by the subjective judgment of the experimenter and cannot be accurately quantified. In addition, the long operation time also reduces the measurement throughput. High performance liquid chromatography (HPLC) and electrochemical methods are time-consuming and labor-intensive, and are limited by equipment. In this regard, some researchers [1] proposed a fluorescent probe method, which not only has high sensitivity and fast detection speed, but also can achieve quantitative detection of nuclease activity. The DNase/RNase fluorescent probe detection kit is one of the best choices.

Detection Method

The method of DNase and RNase Assay kits are the same. Fluorophore-labeled DNA probes or RNA probes are designed and mixed with the test sample.

When the sample does not contain DNases or RNases activity, the probe is stable, the distance between the fluorophore and the quencher group is relatively small, no fluorescent signal is generated due to the principle of fluorescence resonance energy transfer;

When the sample contains DNases or RNases activity, the probe is degraded, the fluorophore and the quencher group move away from each other, resulting in a gradually enhanced fluorescent signal;

The rate of increase in fluorescence signal is positively correlated with the number and activity of the enzyme.

Detection method is shown as following:

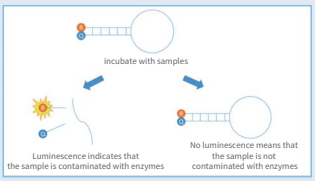


Fig. 1 Schematic diagram of the principle of the fluorescent probe method

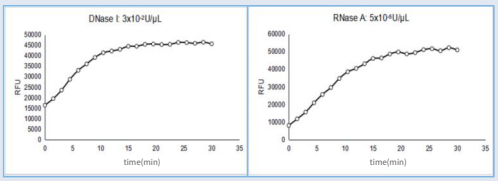


Figure 2. Schematic diagram of the DNase/RNase detection kit reaction curve

Product Pictures

DNASE ASSAY KIT

(FLUORESCENCE)

It is used to detect DNase contamination in the environment and materials in scientific research and production processes.

DNASE (FLUORESCENCE) ASSAY KIT

Application

It is used to detect DNase contamination in the environment and materials in scientific research and production processes.

Characteristics of Products

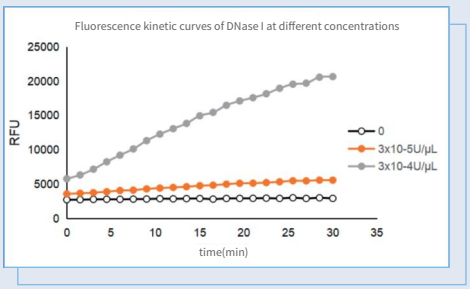
High Sensitivity: Limit of Detection is as low as 1.25×10^{-6} U/μL (DNase I), which is only 1/8 of the best competitor kit.
High precision: intra-batch CV≤10%, inter-batch CV≤15%
Good stability: valid for one year at -20°C. Once the probe solution is prepared, split and stored at -20°C.

Precision and Stability of Hyzmes DNase Assay Kit

Batch number	20220106	20220316	20220425
Inner-batch CV	Low-value sample:3.8% High-value sample:5.4%	Low-value sample:2.1% High-value sample:4.1%	Low-value sample:5.0% High-value sample:9.4%
Inter-batch CV	Low-value sample:3.9% High-value sample:6.5%		
Stability	The probe lyophilized powder is stored at 37°C for 7 days, the deviation from the day 0 meets the requirements; After the probe lyophilized powder was dissolved, it was stored at -20°C for 179 days, the test performance meets the requirements (continuous monitoring).		

Sensitivity higher than gel electrophoresis method

The Hzymes DNase Assay kit was in comparison with the nucleic acid hydrolysis-gel electrophoresis method, the same low-concentration enzyme samples were measured as shown in the figure:

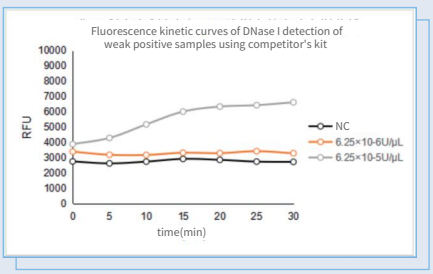


Hyzmes DNase Assay kit measures 3x10^{-5} U/μL DNase I, which can be clearly distinguished from 0

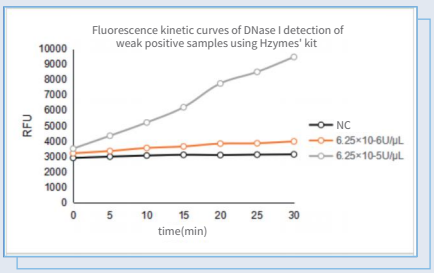
Determination of DNase I by electrophoresis method, No significant difference betwwen 9th/10th channels(3x10^{-5} U/μL) and 1st ~8th channels(≤3x10^{-6} U/μL), 17th channel(negative control)

Sensitivity:Hzymes DNase Assay kit VS competitor kit

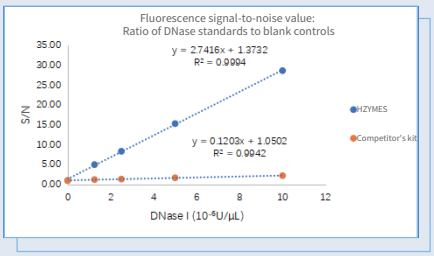
Compared with one of the best competitor kits (fluorescence), the limit of detection of Hzymes DNase Assay kit is only 1/8 of that of the competitor kit.



When the sample concentration is 6.25×10^{-6} U/μL(low-value sample), the signal intensity of the competitor’ s DNase detection kit is indistinguishable from the negative control.



When the sample concentration is 6.25×10^{-6} U/μL(low-value sample), the signal intensity of the Hzymes DNase detection kit is clearly distinguishable from the negative control .



When the sample concentration of DNase I is from 1×10^{-6} to 10×10^{-6} U/μL(low-value sample), the Signal-to-voise(S/N) values of the competitor’ s DNase detection kit is close to 1, however the signal intensity of Hzymes’ DNase Assay Kit is positive correlated to the concentration of DNase I.

Composition of the Hzymes DNase Assay Kit

No.	Name	192T	48T
01	10×reaction solution	2.0mL	0.5mL
02	DNA probe	1 tube	1 tube
03	TE buffer	2.0mL	0.5mL
04	DNase I standard (2U/μL)	20μL	10μL
05	Standard Dilution Buffer	12mL	6mL
06	DNase & RNase-free water	25mL	25mL
07	DNase RNase scavenger	50mL	50mL

Note: The standard is DNase I. The unit for DNase I activity is defined as the amount of enzyme that completely degrades 1μg of pBR322 DNA in DNase I reaction buffer at 37°C for 10 minutes [1]; one DNase I activity unit is equivalent at 0.3 Kunitz units [2].

Equipment and consumables required

Fluorescence microplate reader (including ex/em=485/525nm wavelength)
DNase & RNase-free pipettes and tips
DNase & RNase-free EP tube
DNase & RNase-free black non-transparent 96-well plate

Preparation before experiment (check the user guide for more details)

01.Take out the DNase Assay kit and equilibrate to room temperature (18~25°C), shake and mix the components then centrifuge immediately

02.Centrifuge the DNA probe, add TE buffer to dissolve the powder. Aliquot this DNA probe storage solution; Store at-25~-15°C away from light and avoid repeated freezing and thawing.

03.Set the instrument parameters: Shaking plate 10~15s before detecting; Excitation wavelength λEx=485nm; Emission wavelength λEm=525nm; Temperature 37°C; Endpoint mode.

04.Set the gain to auto-scale if possible, if the microplate reader supports kinetic mode, it is recommended to use the kinetic detection mode.

05.Qualitative detection: select four wells on a 96-well plate: 2μL of DNase I standard solution is diluted with standard dilution Buffer and DNase & RNase-free water to 2×10^{-5} U/μL. Take 80μL as the positive control; 80μL of DNase & RNase-free water as the negative control, 80 μL samples were prepared in duplicate (if insufficient, dilute to 80 μL with DNase & RNase-free water). Add 10 μL DNA probe working solution and 10 μL 10× reaction solution to each well. Read the fluorescence signal value RFU0 immediately; Incubate the plate in the dark at 37°C for 30min, then read the value RFU30 immediately.

If RFU30 (sample to be tested) ⩾ 2×RFU0 (sample to be tested), it is considered that the sample to be tested is contaminated with DNase.

06.Quantitative detection: Take 2μL of DNase I standard solution and dilute it with standard dilution buffer and DNase-free water to 1×10^{-5} U/μL, 5×10^{-6} U/μL, 2.5×10^{-6} U/μL, 1.25×10^{-6} U/μL as standards; DNase & RNase-free water as negative control; follow the same procedures of the qualitative detection, calculate ΔRFU=RFU30-RFU0, with ΔRFU as the ordinate, and the standard DNase I concentration as the abscissa (perform a linear fit, find the fitting equation y=ax+b, put ΔRFU (sample to be tested) into the equation as y, identify x, multiplied by the value of sample pre-dilution, it is the approximate concentration value of the sample to be tested.)

Q&A

Q1：What is the reaction temperature and time of the DNase Assay kit?

Please incubate the reaction system at a constant temperature of 37 °C for 30 minutes.

Q2: When could false positive or false negative results or inaccurate quantitative results occur?

a.Gel buffers, high concentration of viscous substances, surfactants and dark solutions may interfere with the luminescence of fluorophores;

b.If the sample solution to be tested contains substances that inhibit DNase activity, the result of the measurement is the overall enzyme activity of the sample solution, not the activity of the enzymes within it. These substances include:

- High ionic strength solutions (eg. 5M NaCl, 20x SSC, 3M sodium acetate, etc.)
- Buffers with pH<4 or pH>9
- Chaotropic agents, detergents, chelating agents or any solution that denatures proteins (eg. SDS, guanidine thiocyanate, urea, EDTA, etc.)

c.Solutions that cause chemical instability of DNA probes, such as pH>9 solutions, caustic solutions (strong acids and bases, bleach, etc.)

Q3: While preparing standard solutions, in the pre-treatment steps why use the standard dilution buffer instead of DNase & RNase-free water to dilute?

Because the standard is relatively stable in the standard dilution buffer even after series dilution. But If diluted with water,the activity of the standard may change during the series dilution process,resulting deviation of the standard.

Q4: How to adjust the appropriate microplate reader' s gain value?

Select auto-gain option if possible. If your microplate reader does not have auto-gain selection option, first set the middle value according to the gain value range; then adjust the gain value according to the fluorescence signal of the positive control (79 μL DNase & RNase-free water + 1 μL DNase I standard): reduce the gain value if the signal exceeds the upper limit of the instrument; Or increase the gain value appropriatelyif it is far below the upper limit of the instrument .

Q5: Why seriously contaminated samples may lead to false negative results?

The criterion for judging RNase contamination is: RFU30 (sample to be tested) ⩾ 2×RFU0 (sample to be tested), that is, the RFU value at 30 minutes of reaction is more than twice the RFU value at 0 minute. If the sample is seriously contaminated, it is possible that the reaction starts very quickly, so a very high RFU0 value is measured in a very short time, resulting in a false negative result of RFU30 (sample to be tested) < 2×RFU0 (sample to be tested) . At this time, the sample to be tested needs to be diluted with DNase & RNase-free water.

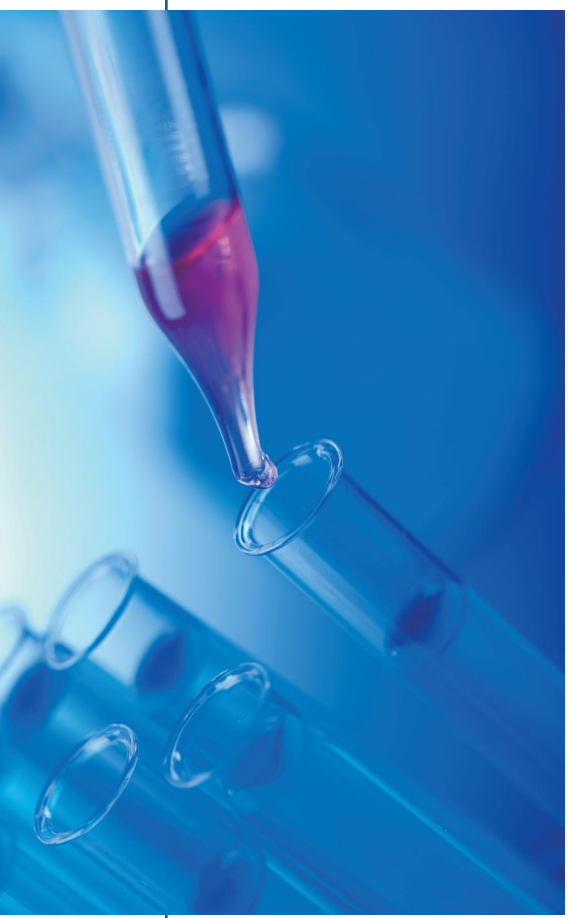
Q6: If the RFU value of the negative control is not 0, does it mean that the negative control is contaminated?

Uncertain. The negative control can also be detected with a low RFU signal (baseline value) but the value will not increase significantly along with the proceeding of the reaction.

Generally, it is considered that the RFU30 is less than twice the RFU0 which indicates that the negative control is not contaminated.

Product details

Catalog Number	Product Name	Specification
HBP002902	DNase assay Kit (Fluorescence)	192 Tests
HBP002903	DNase assay Kit (Fluorescence)	48 Tests



RNASE ASSAY KIT

(FLUORESCENCE)

It is used to detect RNase contamination in the environment and materials in scientific research and production processes.

RNase (FLUORESCENCE) Assay Kit

Application

It is used to detect DNase contamination in the environment and materials in scientific research and production processes.

Characteristics of Products

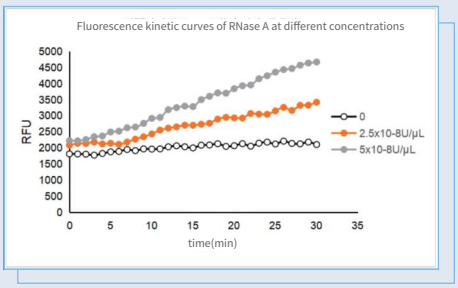
High sensitivity: the detection limit for RNase A is as low as 0.313pg/mL or about 1.56×10^{-9} U/μL which is only 1/8 of the best competitor kit.
High precision: intra-batch CV≤10%, inter-batch CV≤15%
Good stability: valid for one year at -20°C. After the RNA probe solution is prepared, aliquot and store at -20°C.

Precision and Stability of Hyzmes RNase Assay Kit

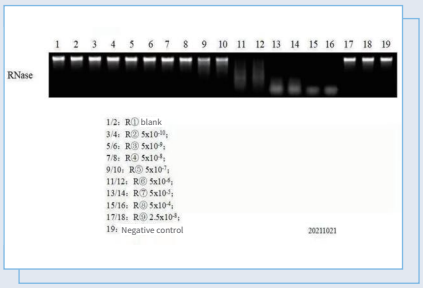
Batch number	20220106	20220316	20220425
Inner-batch CV	Low-value sample:9.4% High-value sample:5.5%	Low-value sample:6.2% High-value sample:6.2%	Low-value sample:8.4% High-value sample:8.3%
Inter-batch CV	Low-value sample:10.3% High-value sample:5.4%		
Stability	The probe lyophilized powder is stored at 37°C for 7 days, the deviation from day 0 meets the requirements; After the probe lyophilized powder was dissolved, it was stored at -20°C for 182 days, the test performance meets the requirements (continuous monitoring)		

Sensitivity higher than gel electrophoresis method

The Hzymes RNase Assay kit was in comparison with the nucleic acid hydrolysis-gel electrophoresis method, the same low-concentration enzyme samples were measured as shown in the figure below.



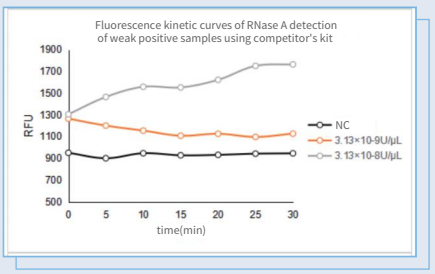
Hyzmes RNase Assay kit measures 2.5x10^{-8} U/μL RNase A, which can be clearly distinguished from 0 concentration



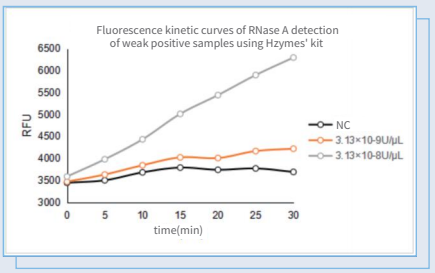
Determination of RNase A by electrophoresis.
No significant difference between 1st ~8th channels (5x10^{-8}U/μL) and 17th , 18th channels (2.5x10^{-8}U/μL), 19th channel(negative control)

Sensitivity:Hzymes RNase Assay kit VS competitor kit

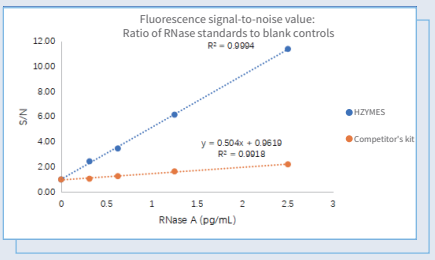
Compared with one of the best comepetitor kits (fluorescence), the limit of detection of Hzymes RNase Assay kit is only 1/8 of that of the competitor kit.



When the sample concentration is 3.13×10^{-9} U/μL(low-value sample), the signal intensity of the competitor’ s RNase detection kit is indistinguishable from the negative control.



When the sample concentration is 3.13×10^{-9} U/μL(low-value sample), the signal intensity of the Hzymes’ RNase Assay Kit is clearly distinguishable from the negative control.



When the sample concentration of RNase A is from 1×10^{-6} to 10×10^{-6} pg/mL, the Signal-to-voise(S/N) values of the competitor’ s DNase detection kit is between 1 to 2, however the signal intensity of Hzymes’ DNase Assay Kit is positive correlated to the concentration of RNase I.

Composition of the Hzymes DNase Assay Kit

No.	Name	192T	48T
01	10×reaction solution	2.0mL	0.5mL
02	RNA probe	1 tube	1 tube
03	TE buffer	2.0mL	0.5mL
04	RNase A standard (2U/μL)	20μL	10μL
05	Standard Dilution Buffer	12mL	6mL
06	DNase & RNase-free water	25mL	25mL
07	DNase RNase scavenger	50mL	50mL

Note: The standard is RNase A. The unit for RNase A activity is defined as the amount of enzyme that increases 1.0 in the absorbance at 260 nm of yeast RNA at pH 5.0 and 37 °C [3], 50 RNase A activity units are equivalent to 1 Kunitz unit [4].

Equipment and consumables required

Fluorescence microplate reader (including ex/em=535/575nm wavelength)
DNase & RNase-free pipettes and tips
DNase & RNase-free EP tube
DNase & RNase-free black non-transparent 96-well plate

Preparation before experiment (check the user guide for more details)

01.Take out the Hzymes RNase Assay kit and equilibrate to room temperature (18~25°C), shake and mix the components then centrifuge immediately.

02.Centrifuge the RNA probe, add TE buffer to dissolve the powder. Aliquot this RNA probe storage solution; store at-25~-15°C away from light and avoid repeated freezing and thawing.

03.Set the instrument parameters: Temperature 37°C, endpoint mode, excitation wavelength/emission wavelength 535/575nm, shaking plate 10~15s before detecting.

04.Set the gain to auto-scale if possible, if the microplate reader supports kinetic detection mode, it is recommended to use the kinetic detection mode.

05.Qualitative detection: select four wells on a 96-well plate: 2μL of RNase A standard solution (10mg/mL) is diluted with standard dilution Buffer and DNase & RNase-free water to 5pg/mL (2.5×10^{-8} U/μL).Take 80μLas the positive control; 80μL of DNase & RNase-free water as the negative control, 80 μL samples were prepared in duplicate (if insufficient, dilute to 80 μL with DNase & RNase-free water). Add 10 μL RNA probe working solution and 10 μL 10×reaction solution to each well. Read the fluorescence signal value RFU0 immediately; Incubate the plate in the dark at 37°C for 30min, then read the value RFU30 immediately

If RFU30 (sample to be tested) ⩾ 2×RFU0 (sample to be tested), it is considered that the sample to be tested is contaminated with RNase.

06.Quantitative detection: Take 2μL of RNase A standard solution (10mg/mL) and dilute it with standard dilution buffer and DNase-free water to 2.5×10^{-9} mg/μL, 1.25×10^{-9} mg/mL, 6.25×10^{-10}mg/mL, 3.13×10^{-10} mg/mL as standards; DNase & RNase-free water as the negative control; follow the same procedures of the qualitative detection, calculate ΔRFU=RFU30-RFU0, with ΔRFU as the ordinate, and the standard RNase A concentration as the abscissa (perform a linear fit, find the fitting equation y=ax+b, put ΔRFU (sample to be tested) into the equation as y, identify x, multiplied by the value of sample pre-dilution, it is the approximate concentration value of the sample to be tested.)

Q&A

Q1: What is the reaction temperature and time of the RNase Assay kit?

Please incubate the reaction system at a constant temperature of 37 °C for 30 minutes.

Q2 : When could false positive or false negative results or inaccurate quantitative results occur?

a.Gel buffers, high concentration of viscous substances, surfactants and dark solutions may interfere with the luminescence of fluorophores;

b.If the sample solution to be tested contains substances that inhibit DNase activity, the result of the measurement is the overall enzyme activity of the sample solution, not the activity of the enzymes within it. These substances include:

- High ionic strength solutions (eg. 5M NaCl, 20x SSC, 3M sodium acetate, etc.)
- Buffers with pH<4 or pH>9
- Chaotropic agents, detergents, chelating agents or any solution that denatures proteins (eg. SDS, guanidine thiocyanate, urea, EDTA, etc.)

c.Solutions that cause chemical instability of DNA probes, such as pH>9 solutions, caustic solutions (strong acids and bases, bleach, etc.)

Q3：While preparing standard solutions, in the pre-treatment steps why use the standard dilution buffer instead of DNase & RNase-free water to dilute?

Because the standard is relatively stable in the standard dilution buffer even after series dilution. But If diluted with water, the activity of the standard may change during the series dilution process.,resulting deviation of the standard .

Q4: How to adjust the appropriate microplate reader gain value?

Select auto-gain option if possible. If your microplate reader does not have auto-gain selection option, first set the middle value according to the gain value range; then adjust the gain value according to the fluorescence signal of the positive control (79 μL DNase & RNase-free water + 1 μL DNase I standard): reduce the gain value if the signal exceeds the upper limit of the instrument; Or increase the gain value appropriatelyif it is far below the upper limit of the instrument .

Q5: Why could a heavily contaminated sample lead to false negative results?

he criterion for judging RNase contamination is: RFU30 (sample to be tested) ⩾ 2×RFU0 (sample to be tested), that is, the RFU value at 30 minutes of reaction is more than twice the RFU value at 0 minute. If the sample is seriously contaminated, it is possible that the reaction starts very quickly, so a very high RFU0 value is measured in a very short time, resulting in a false negative result of RFU30 (sample to be tested) < 2×RFU0 (sample to be tested) . At this time, the sample to be tested needs to be diluted with DNase & RNase-free water.

Q6: If the RFU value of the negative control is not 0, does it mean that the negative control is contaminated?

uncertain. The negative control can also be detected with a low RFU signal (baseline value) but the value will not increase significantly along with the proceeding of the reaction.

Generally, it is considered that the RFU30 is less than twice the RFU0 which indicates that the negative control is not contaminated.

Product details

Catalog Number	Product Name	Specification
HBP003002	RNase assay Kit (Fluorescence)	192 Tests
HBP003003	RNase assay Kit (Fluorescence)	48 Tests

References

[1]New England Biolabs DNase I User Guide

[2]Kunitz M. Crystalline Desoxyribo - nuclease I. Isolation and General Pro - perties Spectrophotometric Method for the Measurement of Desoxyribonuclease Activity[J]. The Journal of General Physiology, 1950, 33(4):349-362.

[3]Thermo scientific RNase A(DNase and Protease-free) User Guide

[4]Kunitz, M. A. A spectrophotometric method for the measurement of ribonuclease activity[J]. Journal of Biological Chemistry, 1946, 3(2):308-320.