

DNase Detection Kit

Fluorescence based detection of DNase activity

Contamination Control Kits

PP-402S	20 reactions
PP-402L	100 reactions

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20 °C, avoid frequent thawing and freezing

Fluorescent DNase probe must be stored in the dark

Store DNase standards in a separate box

Description

The DNase Detection Kit provides optimized reagents for a highly sensitive, fast and easy-to-perform DNase detection assay. The kit allows the detection of lowest amounts of DNase I and other ss- or ds-DNA degrading enzymes. It is the ideal tool for contamination testing of a few samples or for routine process monitoring.

The kit is based on a fluorescent probe exhibiting minimal fluorescence but showing a strong increase in fluorescence in the presence of DNases. The probe is linked to the fluorophore FAM as reporter dye allowing excitation and detection with nearly all common real-time PCR cyclers or fluorescence readers.

Using the DNase Detection Kit as little as $5 \cdot 10^{-3}$ units (approx. 10 pg) of DNase I can be detected within 20-30 min.

Please note: DNase containing vials should be stored in a separate box / on a separate place and opened only in a separate lab area to avoid DNase contamination of other reagents!

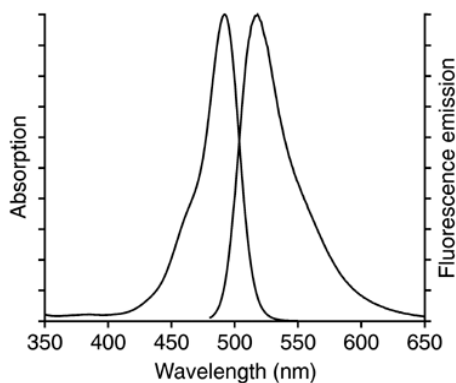
ROX reference dye

ROX reference dye does not take part in the detection reaction and allows therefore, a normalization for non-DNase related signal variations. We recommend additional ROX as internal standard if instruments compatible with the evaluation of the ROX reference signal are used. The ROX dye has an emission maximum at 601 nm.

Spectroscopic data of FAM

Excitation maximum: $\lambda_{Ex} = 495$ nm

Emission maximum: $\lambda_{Em} = 520$ nm



FAM excitation and emission spectra

Fluorescent DNase probe (red cap)

Detection Buffer (green cap)

10x concentration

DNase-free water (white cap)

ROX reference dye (purple cap)

50x concentration

DNase standard I (yellow cap)

$0.5 \cdot 10^{-3}$ units/ μ l (approx. 1 pg/ μ l)

DNase standard II (yellow cap)

$5 \cdot 10^{-3}$ units/ μ l (approx. 10 pg/ μ l)

DNase standard III (yellow cap)

$50 \cdot 10^{-3}$ units/ μ l (approx. 100 pg/ μ l)

Preparation of the detection assay

Prepare 13 volumes of master mix for 12 samples or a triple-set of 4 samples. Pipet with sterile filter tips, use DNase free tubes / plates and minimize the exposure of the fluorescent DNase probe and the master mix to light. Perform the setup in a DNase-free area. No-template controls and a dilution series of DNase as standards should be included in each assay. Prepare the following master mix:

		stock conc.	final conc.	20 μ l rxns	50 μ l rxns
Detection Buffer	red cap	10x	1x	2 μ l	5 μ l
Fluorescent DNase probe	red cap			0.4 μ l	1 μ l
ROX reference dye *	purple cap	50x	1x	0.4 μ l	1 μ l
DNase-free water	white cap			7.6 μ l	34 μ l
<i>total</i>				10 μ l	40 μ l

* ROX reference dye increases the accuracy of measurement if used with a machine that is compatible with the evaluation of the ROX reference signal.

Data collection

Real-time fluorescence measurements

This detection method allows a kinetic evaluation of DNase activity resulting in an increased accuracy and detection limit.

Dispensing the master mix

Vortex the master mix thoroughly to assure homogeneity. Dispense 10 μ l or 40 μ l, respectively into each vial or well if using a real-time PCR plate.

Addition of the sample

The addition of the samples to the master mixes must be performed on ice. Add the DNase standards in a separate area to avoid DNase contamination of other reagents!

Add 10 μ l of each sample or 10 μ l of DNase-free water as no-template control or 10 μ l of the DNase standards to the wells containing the master mix. Cap or seal the vials / plate. Centrifuge to assure homogeneity and to remove possible bubbles before placing the samples in the real-time fluorescence reader.

Plate Configuration

	1	2	3	4	5	6	7	8	9	10	11	12
A	10 μ l / 40 μ l master mix + 10 μ l DNase-free water (neg. control)			10 μ l / 40 μ l master mix + 10 μ l DNase standard I (0.005 units)			10 μ l / 40 μ l master mix + 10 μ l DNase standard II (0.05 units)			10 μ l / 40 μ l master mix + 10 μ l DNase standard III (0.5 units)		
B	10 μ l / 40 μ l master mix + 10 μ l Sample 1			10 μ l / 40 μ l master mix + 10 μ l Sample 2			10 μ l / 40 μ l master mix + 10 μ l Sample 3			10 μ l / 40 μ l master mix + 10 μ l Sample 4		
C	10 μ l / 40 μ l master mix + 10 μ l Sample 5			10 μ l / 40 μ l master mix + 10 μ l Sample 6			10 μ l / 40 μ l master mix + 10 μ l Sample 7			10 μ l / 40 μ l master mix + 10 μ l Sample 8		
D	10 μ l / 40 μ l master mix + 10 μ l Sample 9			10 μ l / 40 μ l master mix + 10 μ l Sample 10			10 μ l / 40 μ l master mix + 10 μ l Sample 11			10 μ l / 40 μ l master mix + 10 μ l Sample 12		
E	etc.			etc.			etc.			etc.		
F	etc.			etc.			etc.			etc.		
G	etc.			etc.			etc.			etc.		
H	etc.			etc.			etc.			etc.		

Recommended incubation and detection

Incubation	37°C	1 min	20-30x
Fluorescence detection			

If using a real-time PCR cyclor for data collection the set-up program may not accept incubation terminated by fluorescence detection. In that case the following modified two-step cycling protocol is recommended:

1. Incubation	36°C	10 sec	20-30x
2. Incubation	37°C	50 sec	
Fluorescence detection			

Endpoint measurement using a fluorometer

This detection method allows only the end point determination of the accumulated fluorescence of each sample. Please note that this method will limit the accuracy and detection limit.

Dispensing the master mix

Vortex the master mix thoroughly to assure homogeneity. Dispense 10 µl or 40 µl, respectively into reaction tubes.

Addition of the sample

Add the DNase standards in a separate area to avoid DNase contamination of other reagents!

Add 10 µl of each sample or 10 µl of DNase-free water as no-template control or 10 µl of the DNase standards to the tubes containing the master mix. Cap or seal the tubes. Centrifuge the tubes to assure homogeneity before placing them in a thermoblock.

Recommended incubation

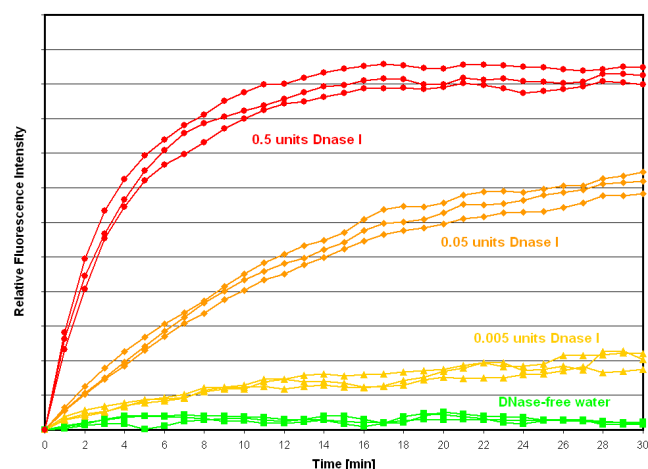
Incubate the tubes for 20-30 min at 37°C.

Fluorescence detection

Transfer the reaction mixtures into the vials of a fluorescence reader plate and place it in the reader. Start the measurement.

Analysis of the measurement

Real-time plot of the fluorescence data



Kinetic evaluation of DNase I activity monitored on a real-time fluorescence reader. 0.005 units DNase I are equivalent to approx. 10 pg.

Detection Limit

Amounts of $5 \cdot 10^{-3}$ units (approx. 10 pg) of DNase I per tube can be usually detected. The limit of detection (LOD) is defined as 3x standard deviation of the negative control.

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