Data Sheet

IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Jena Bioscience

DNase+RNAse Detection Kit

Fluorescence based detection of DNase and RNase activities

Contamination Control Kits

	Cat.-No.	Amount
	PP-407S	20 reactions
	PP-407L	100 reactions

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C, avoid frequent thawing and freezing
Fluorescent DNase+RNase probe must be stored in the dark
Store DNase+RNase standards in a separate box

Fluorescent DNase+RNAase Probe (red cap)

Detection Buffer (green cap)
10x concentration

Nuclease-free Water (white cap)

ROX Reference Dye (purple cap)
50x concentration

DNase+RNase Standard low (yellow cap)
0.2 munits/µl (approx. 0.4 pg/µl) DNase
0.02 pg/µl RNase A

DNase+RNase Standard mid (yellow cap)
2 munits/µl (approx. 4 pg/µl) DNase
0.2 pg/µl RNase A

DNase+RNase Standard high (yellow cap)
20 munits/µl (approx. 40 pg/µl) DNase
2 pg/µl RNase A

Description
RNase+DNase Detection Kit provides optimized reagents for highly sensitive, fast and easy-to-perform DNase and RNase single step detection assay. The kit allows the detection of lowest amounts of RNase A, RNase T1, RNase 1 and other RNA degrading enzymes as well as DNase I and other ss- or ds-DNA degrading enzymes. It is ideal for contamination testing of a few samples as well as for routine process monitoring.
The performance of the kit is based on two fluorescent probes exhibiting minimal fluorescence but showing a strong increase in fluorescence in the presence of DNases or RNases, respectively. The DNase probe is linked to the fluorophore FAM and the RNase probe to JOE as reporter dyes allowing excitation and detection with nearly all common real-time PCR cyclers or end-point readers.
Using the DNase+RNase Detection Kit as little as 0.2 pg RNae A and 2 munits (approx. 4 pg) of DNase I can be detected within 20-30 min.
Please note: DNase+RNase containing vials should be stored in a separate box / on a separate place and opened only in a separate lab area to avoid DNase and RNase contamination of lab space or 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 (DNase Probe)
Excitation maximum: λ_{Ex} = 495 nm
Emission maximum: λ_{Em} = 520 nm

Spectroscopic data of JOE (RNase Probe)
Excitation maximum: λ_{Ex} = 520 nm
Emission maximum: λ_{Em} = 548 nm

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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+RNAse probe and the master mix to light. Perform the setup in a DNase- and RNase-free area. No-template controls and a dilution series of DNase and RNase as standards should be included in each assay. Prepare the following master mix:

		stock conc.	**final conc.**	**20 µl rnxs**	**50 µl rnxs**
Detection Buffer	red cap	10x	1x	2 µl	5 µl
Fluorescent DNase+RNase probe	red cap			0.4 µl	1 µl
*ROX reference dye **	*purple cap*	*50x*	*1x*	*0.4 µl*	*1 µl*
nuclease-free water	white cap			7.6 µl	34 µl
total				*10 µl*	*40 µl*

* 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 and RNase activities resulting in an increased accuracy and detection limit.

Dispensing the master mix

Vortex the master mix thoroughly to assure homogeneity. Dispense 10 µl or 40 µl, 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+RNAse standards in a separate area to avoid DNase and RNAse contamination of other reagents and lab space!

Add 10 µl of each sample or 10 µl of nuclease-free water as no-template control or 10 µl of the DNase+RNase 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 µl / 40 µl master mix + 10 µl DNase-free water (neg. control)			10 µl / 40 µl master mix + 10 µl DNase standard I (0.005 units)			10 µl / 40 µl master mix + 10 µl DNase standard II (0.05 units)			10 µl / 40 µl master mix + 10 µl DNase standard III (0.5 units)		
B	10 µl / 40 µl master mix + 10 µl Sample 1			10 µl / 40 µl master mix + 10 µl Sample 2			10 µl / 40 µl master mix + 10 µl Sample 3			10 µl / 40 µl master mix + 10 µl Sample 4		
C	10 µl / 40 µl master mix + 10 µl Sample 5			10 µl / 40 µl master mix + 10 µl Sample 6			10 µl / 40 µl master mix + 10 µl Sample 7			10 µl / 40 µl master mix + 10 µl Sample 8		
D	10 µl / 40 µl master mix + 10 µl Sample 9			10 µl / 40 µl master mix + 10 µl Sample 10			10 µl / 40 µl master mix + 10 µl Sample 11			10 µl / 40 µl master mix + 10 µl 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 cycler 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			

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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+RNAse standards in a separate area to avoid DNase and RNAse contamination of other reagents and lab space!

Add 10 µl of each sample or 10 µl of nuclease-free water as no-template control or 10 µl of the DNase+RNase 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

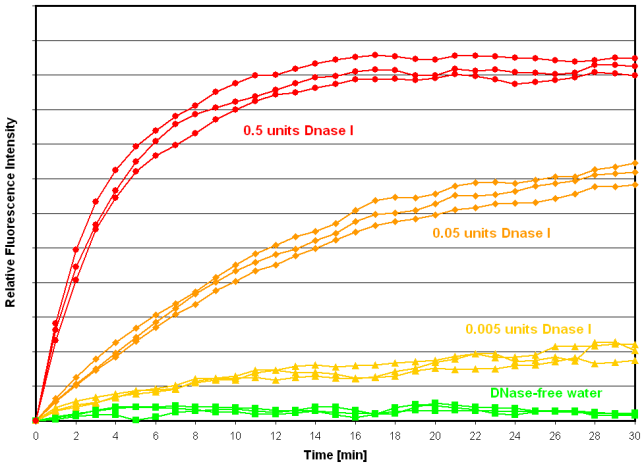
Incubate the tubes for 20-30 min at 37°C.

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

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