Data Sheet

Jena Bioscience
Building Blocks of Life

RNase+DNase Detection Kit

Fluorescence based detection of RNase and DNase activity

Contamination Control Kit

Cat. No.	Amount
PP-409S	100 reactions
PP-409L	500 reactions

For general laboratory use

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C, store in the dark, avoid freeze/thaw cycles

Additional Storage Conditions: stable at 4 °C for up to 1 week

Shelf Life: 12 months

Form: liquid

Content

Component	Cap	100 reactions	500 reactions
RNase+DNase Detection Master [1] 2 x conc	red	1 ml	5 x 1 ml
ROX Reference Dye 25 µM / 50 x	purple	50 µl	5 x 50 µl
RNase+DNase Standard 200 pg/µl RNase A 0.02 units/µl DNase I	yellow	10 µl	3 x 10 µl
Dilution Buffer 1 x conc	blue	3 x 1.8 ml	9 x 1.8 ml
PCR-grade Water	white	1.2 ml	1.2 ml

[1] Consider reasonable aliquotation of the detection master mix to avoid freeze / thaw cycles

Required measuring device

Real-time PCR cycler (recommended) or fluorescence spectrometer

Description

The RNase+DNase Detection Kit provides a highly sensitive, fast and easy-to perform multiplex system for parallel detection of RNase and DNase activity. The kit allows the detection of lowest amounts of RNase and ss- or ds-DNA degrading DNases. It is the ideal tool for contamination testing, ranging from a few samples to routine process monitoring.
The detection kit is based on a combination of fluorescently labeled RNA and DNA probes. Both probes exhibit minimal fluorescence but show a strong increase in fluorescence intensity in the presence of RNases and DNases, respectively. The RNA probe is linked to fluorophore FAM as reporter dye, the DNA probe is linked to JOE allowing excitation and detection with nearly all common real-time PCR cyclers or fluorescence readers.
Please note: Opened RNase or DNase containing vials should be stored in a separate box / on a separate place and opened only in a separate lab area to avoid RNase or DNase contamination of other reagents!

Detection limit

The detection limit of the assay is
RNase A: < 0.1 pg/µl
DNase I: < 1 x 10^{-5} units/µl

ROX reference dye

ROX Reference Dye does not take part in the detection reaction and allows therefore a normalization for non-RNase or -DNase related signal variations. We recommend to add ROX as internal standard if the instrument is compatible with the evaluation of the ROX reference signal.

Spectroscopic data of FAM (RNase Probe)

Excitation maximum: λ_{Ex} = 495 nm
Emission maximum: λ_{Em} = 520 nm

Spectroscopic data of JOE (DNase Probe)

Excitation maximum: λ_{Ex} = 520 nm

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Emission maximum: λ_{Em} = 548 nm
Use the filter set for VIC if a filter for JOE is not available.

Spectroscopic data of ROX (internal reference)

Excitation maximum: λ_{Ex} = 576 nm
Emission maximum: λ_{Em} = 601 nm

Preparation of Samples

A sample volume of 10 µl per assay is recommended. Samples containing high concentrations of salt, highly viscous liquids or detergents should be diluted with PCR-grade water before testing. Enzymes, buffers or other components with a high concentration of glycerol must be diluted with PCR-grade water to make sure that the final glycerol concentration in the detection assay does not exceed 2%. Please note that dilution of the sample decreases the final assay sensitivity.
Samples that contain fluorophores or fluorescence quenching components may interfere with the fluorophore-quencher based analyzing method and are not recommended for testing with this kit.

Preparation of RNase+DNase Standard

Dilute the provided ***RNase+DNase Standard*** (200 pg/µl RNase A, 0.02 units/µl DNase I) with ***Dilution Buffer*** in a ratio of 1:500 to obtain:

- ***Standard high*** (0.4 pg/µl RNase A, 4 x 10^{-5} units/µl DNase I)

Dilute the ***Standard high*** with ***Dilution Buffer*** in a ratio of 1:4 to obtain:

- ***Standard low*** (0.1 pg/µl RNase A, 1 x 10^{-5} units/µl DNase I)

Preparation of individual standard concentrations is possible if required.

Preparation of the detection assay

Pipet with sterile filter tips, use RNase/DNase free tubes / plates and minimize the exposure of the master mix to light. Perform the setup in a RNase/DNase-free area. No-template controls and a dilution series of RNase+DNase standards should be included in each test series. Measuring all samples and standards in triplets is highly recommended.
ROX Reference Dye increases the accuracy of measurement if working with a qPCR cycler or spectrometer that is compatible with the evaluation of the ROX reference signal.
Add ROX Reference Dye (25 µM, 50 x conc.) to RNase+DNase Detection Master (2 x conc.) as following:

Component	Conc	20 reactions	100 reactions	500 reactions
RNase+ DNase Detection Master	2 x	200 µl	1 ml	5 x 1 ml
ROX Reference Dye	50 x	8 µl	40 µl	5 x 40 µl

Preparation of the assay on ice is recommended to obtain strong and reproducible signal yields. A final assay volume of 20 µl is recommended.
Add the RNase/DNase standards in a separate area to avoid DNase contamination of other samples or reagents!

- Mix carefully **by pipetting** to assure homogeneity of RNase+DNase Detection Master
- Add 10 µl RNase+DNase Detection Master into each PCR tube / well of the PCR plate
- Add 10 µl PCR-grade water to each tube for negative controls and close the tubes
- Add 10 µl of sample material to each sample tube and close the tubes
- Add 10 µl Standard low to each tube for low standards and close the tubes
- Add 10 µl Standard high to each tube for high standards and close the tubes
- Spin down and **make sure to avoid vortexing / formation of air bubbles**

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- Place the tubes in the qPCR cycler or spectrometer

	1	2	3	4	5	6	7	8	9	10	11	12
A	neg. control			standard low			standard high			sample 1		
B	sample 2			sample 3			sample 4			sample 5		
C	sample 6											
D												
E												
F												
G												
H												

Pipetting schema of a 96-well plate. Measuring all samples and standards in triplets is highly recommended.

Data collection

Kinetic measurement

A real-time fluorescence measurement allows a kinetic evaluation of RNase and DNase activity resulting in an increased accuracy and better detection limit.
The following incubation and detection sequence is recommended:

Incubation	37°C	1 min	20-30 x
Fluorescence data collection in FAM channel, JOE/VIC channel and ROX channel (if using ROX)			

If using an older real-time PCR cycler for data collection the set-up program may not accept incubation at constant temperature terminated by fluorescence detection. In that case the following "two-step" cycling protocol is recommended:

Incubation	36°C	10 sec	20-30 x
Incubation	37°C	50 sec	
Fluorescence data collection in FAM channel, JOE/VIC channel and ROX channel (if using ROX)			

Endpoint measurement using a fluorometer/spectrometer

This detection method allows only the end point determination of the accumulated fluorescence signal of each sample. Please note that this method may limit accuracy and detection limit. It is essential to avoid measurements in the flattened area at the end of the fluorescence curve that may occur if incubation time exceeds 20 min.
Incubate the tubes for 10-20 min at 37°C. Measure both, FAM fluorescence signal and JOE fluorescence signal for each sample in a fluorescence reader or spectrometer.

Analysis of the measurement

Kinetic measurement on a real-time PCR system

After finishing the measurement switch to the ***Results*** area, select ***Amplification Plot*** and **ΔRn vs Cycle** as Plot Type. Select ***Linear*** as Graph Type. Switch off ***Automatic Baseline*** and select ***Baseline Start Cycle 1*** and ***End Cycle 1***.
The resulting plot shows the relative fluorescence intensities vs. time in linear scale. The plot is normalized to the first data point at time 0.
Select the FAM channel: The slope of the plot in its linear region is directly proportional to the RNase activity.
Select the JOE or VIC channel: The slope of the plot in its linear region is directly proportional to the DNase activity.

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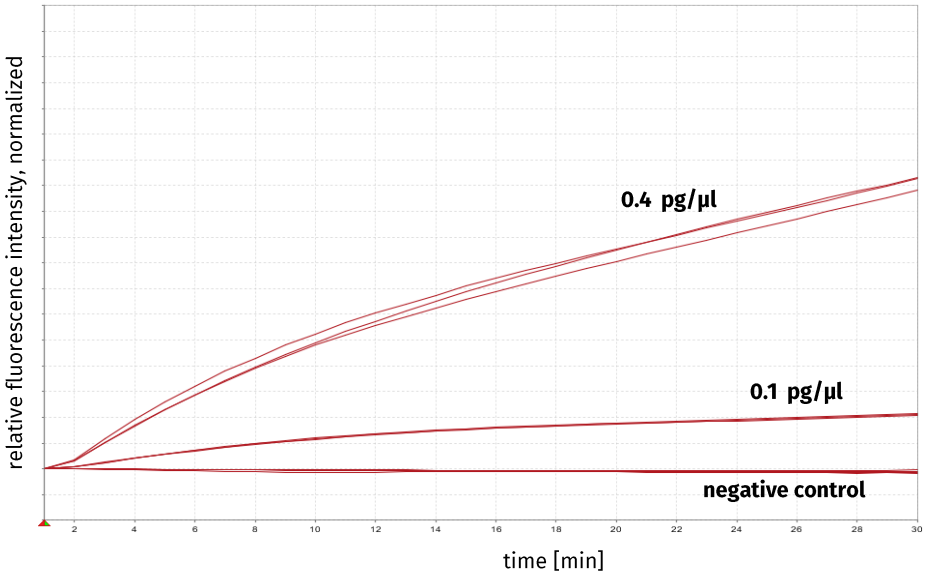
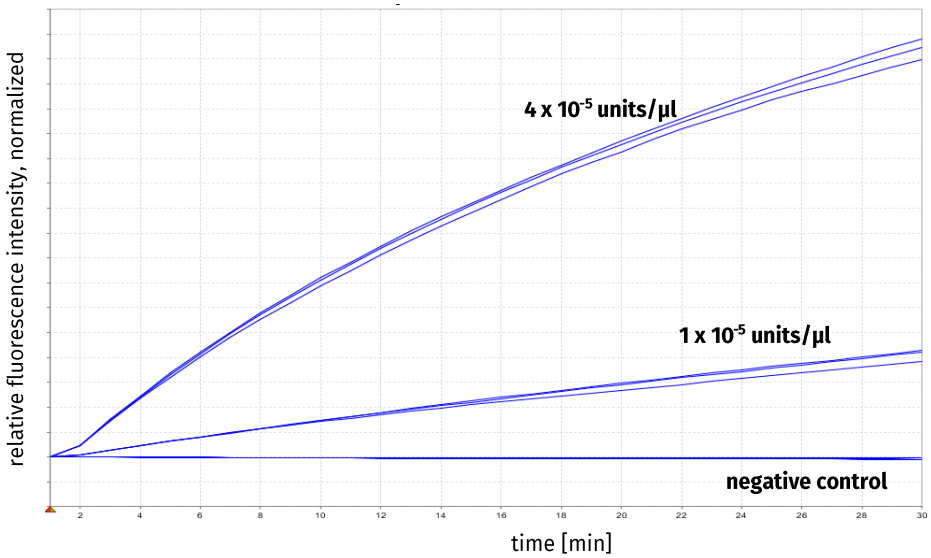


Kinetic evaluation of RNase A activity monitored on the real-time PCR system QuantStudio 5 (ThermoFisher). Concentration of RNase A standards are 0.1 pg/µl and 0.4 pg/µl. PCR-grade water is used for negative control.



Kinetic evaluation of DNase I activity monitored on the real-time PCR system QuantStudio 5 (ThermoFisher). Concentration of DNase I standards are 1 x 10^{-5} units/µl and 4 x 10^{-5} units/µl. PCR-grade water is used for negative control.