

RNase+DNase Detection Kit

Cat #: orb1733619 (manual)

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	Сар	100 reactions	500 reactions
RNase+DNase Detection Master 2 x cone	red	1 ml	5 x 1 ml
ROX Reference Dye 25 μ M / 50 X	purple	50 µ1	5 x 50 µl
RNase+DNase Standard 200 pg/µl RNase A 0.02 units/µl DNase I	yellow	10 µ1	3 x 10 µl
Dilution Buffer 1 x cone	blue	3 x 1.8 ml	9 x 1.8 ml
PCR-grade Water	white	1.2 ml	1.2 ml

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: $< 0.1 \text{ pg/}\mu l$ DNase I: $< 1 \times 10^{-5} \text{ units/}\mu l$

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: $\lambda_{Ex} = 495$ nm Emission maximum: $\lambda_{Em} = 520$ nm

Spectroscopic data of JOE (DNase Probe)

Excitation maximum: $\lambda_{Ex} = 520$ nm Emission maximum: $\lambda_{Em} = 548$ nm Use the filter set for VIC if a filter for JOE is not available.

Spectroscopic data of ROX (internal reference)

Excitation maximum: $\lambda_{Ex} = 576$ nm Emission maximum: $\lambda_{Em} = 601$ 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/ μ l RNase A, 4 × 10⁻⁵ units/ μ l DNase I)

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

• Standard low (0.1 pg/ μ l RNase A, 1 × 10⁻⁵ units/ μ l DNase I)

Preparation of individual standard concentrations is possible if required.



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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 μ M, 50 x cone.) to RNase+DNase Detection Master (2 x cone.) as following:

Component	Cone	20 reactions	100 reactions	500 reactions
RNase+ DNase Detection Master	2x	200 µl	1 ml	5 x 1 ml
ROX Reference Dye	50 X	8 µ1	40 µ1	5 x 40 µ1

Preparation of the assay on ice is recommended to obtain strong and reproducible signal yields. A final assay volume of $20 \ \mu l$ 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 μ I 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
- Place the tubes in the qPCR cycler or spectrometer

	1	2	3	4	5	6	7	8	9	10	11	12
Α	n	eg. contr	ol	standard low		standard high		sample 1				
В		Sample 2	2	Sample 3		Sample 4		Sample 5				
С		Sample 6	5									
D												
E												
F												
G												
Н												



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

Data collection

Kinetic measurement

A real-time florescence 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	20.20
Fluorescence data colle	20-30 X		

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	
Incubation	37°C	50 sec	20-30 x
Fluorescence data colle			

Endpoint measurement using a fuorometer/spectrometer

This detection method allows only the end point determination of the accumulated fluorescence signal of each sample. Please note that this method maylimit 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 \triangle **Rn 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 VC channel: The slope of the plot in its linear region is directly proportional to the DNase activity.





Kinetic evaluation of RNase A activity monitored on the real-time PCR system QuantStudio 5 (ThermoFisher). Concentration of RNase A standards are 0.1 $pg/\mu l$ and 0.4 $pg/\mu l$. 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 1×10^{-5} units/µl and 4×10^{-5} units/µl. PCR- grade water is used for negative control.