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Clara™ Probe 1-Step Purple Mix Separate-ROX

Product description

Clara™ Probe 1-Step Purple Mix offers reliable probe-based qPCR detection of both RNA and DNA target sequences. Provided in a one-tube format, this poweful RT-qPCR mix gives superior target amplification, in single or multiplex assays, even from highly dilute samples.

Clara™ Probe 1-Step Purple Mix is a 4x qPCR mix containing hot start Taq polymerase, dNTPs, $MgCl_2$, an enhanced version of UltraScript™ Reverse Transcriptase, and our RiboShield™ RNase inhibitor, providing a complete 1-step RT-qPCR mix. It is developed to work well with the full range of probe types, including TaqMan®, Scorpions® and molecular beacons and can be used both for diagnostic and basic research puproses. A separate tube of 50 µM ROX additive is provided, enabling use on all real time instruments.

The mix contains an inert purple dye to aid sample visualisation during manual plate setup and in high-throughput workflows. This dye is non-inhibitory to PCR and does not affect reaction efficiency and sensitivity. Depending on the chosen probe fluorophore, some quenching of fluorescence intensity may be observed.

Our extensive optimisation makes this mix suitable for all nucleic target types. We have tested it against common RNA viruses, including SARS-CoV-2, RSV, Influenza A, and B, standard housekeeping genes, such as g-actin and GAPDH, as well as DNA targets.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

Pack size	4x Clara™ Probe 1-Step Purple Mix No-ROX	50 µM ROX Additive
200 reactions	1 x 1 mL	1 x 200 µL
600 reactions	3 x 1 mL	1 x 200 µL
1000 reactions	5 x 1 mL	1 x 200 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -15 °C. Avoid prolonged exposure to light. If stored correctly, the kit will retain full activity until the indicated expiry date. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum.

Limitations of product use

For research use only.

Technical support

Help and support are available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting please email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

Important considerations

Instrument compatibility: This mix is suitable for all ROX requirements. Different real-time PCR instruments require different levels of ROX passive reference. Please use our qPCRBIO Selection Tool to determine which ROX concentration your instrument requires (https://pcrbio.com/resources/qpcr-selection-tool/).

Template: The kit can be used with RNA or DNA extracted by most commercial kits or standard extraction methods, provided the amount and quality of template are within an acceptable range. Addition of 2 to 5 µL volumes of sample will improve assay precision.

ROX additive protocol: The 50 µM ROX Additive supplied is formulated to be added directly to the tube or bottle of 4x qPCR mix supplied. Once the ROX is added, the reagent may be used straight away or stored between -30 ˚C and -15 ˚C for future use. Please use the table in "Reaction setup" below to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Probe Intensity: The purple dye in Clara™ Probe Purple Mix may reduce fluorescence intensity from probes by absorbing light at both the excitation and emission wavelengths (see Table 1). However, the recomended probe concentration prove sufficient for detection on all instruments tested. If signal intensity is a concern, consider switching to a Clara™ Probe Mix without dye.

Table 1: Fluorescent intensity of selected probes in Clara™ Probe 1-Step Purple Mix.

Fluorophore	Ex / Em (nm)	Signal loss
FAM	494 / 518	25%
HEX	535 / 556	30%
Texas Red	595 / 615	25%
Cy5	675 / 694	10%

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara™ Probe 1-Step Mix No-ROX, add ROX as required.

Hi-ROX instruments	Lo-ROX instruments	Hi-ROX instruments	Reaction concentration
4x Clara™ Probe 1-Step Mix No-ROX	1.0 mL	1 mL	1x
50 µM ROX Additive	4.0 µL	40.0 µL	50 nM (Lo-) , 500 nM (Hi-ROX)

2. Prepare a master mix based on the following table.

Reagent	20 µL reaction	Final concentration	Notes
4x Clara™ Probe 1-Step Purple Mix	5 µL	1x	Ensure ROX is added prior to this step.
Forward primer (0.1 - 1 mM)	1-2 µL	400 nM - 1 µM	
Reverse primer (0.1 - 1 mM)	1-2 µL	400 nM - 1 µM	
Probe (0.1 - 1 mM)	0.25-1 µL	125 - 500 nM	
RNA or DNA Template	2-5 µL	Variable	<100 ng cDNA, <1 µg genomic DNA, 1 pg-1 µg total RNA, >0.01 pg mRNA, 4 to 1x10^8 copies viral RNA
PCR grade dH_2O	Up to 20 µL final volume		

3. Program the instrument using the following conditions, acquiring data on the appropriate channel(s) for your chosen probe(s):

Cycles	Temperature General	Time	Notes
1 *Optional*	52 ˚C	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription. Required only for RNA templates.
1	95 ˚C	3 minutes	Polymerase activation and RTase inactivation
40-50	95 ˚C 55 ˚C-65 ˚C	5-15 seconds 20-30 seconds	Denaturation Anneal/Extension
Melt analysis	Refer to instrument instructions		Optional melt profile analysis, available for hybridisation probes only