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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 RT-qPCR mix gives superior target amplification, in single or multiplex assays, from even 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.

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.

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 -20 °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.

Technical support

Scan or click the QR code for troubleshooting help and answers to frequently asked technical questions. If you require further technical support, please email technical@pcrbio.com with the following information:

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

TROUBLESHOOT

FAQS

Important considerations

Instrument compatibility: Different qPCR instruments may require different levels of ROX passive reference for normalisation. Use our qPCR Selection Tool to determine which ROX concentration your instrument requires (https://pcrbio.com/resources/qpcr-selection-tool/).

ROX additive: The 50µM ROX Additive can be added directly to the 1 mL tube of mix supplied. Once added, the reagent may be used straight away or stored at -30 ˚C to -20 ˚C for future use. Use the table below to add the correct amount of ROX for your instrument. Vortex thoroughly after ROX addition.

Component	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)

Template: The mix 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.

Primer design: For efficient amplification we recommend amplicon lengths between 80-200 bp and not exceeding 400 bp. Shorter amplicons allow for faster cycling. Primers should have an approximate Tm of ~60 ˚C using default Primer 3 settings (https://bioinfo.ut.ee/primer3/). To verify the best annealing temperature for your primers in our products, please visit https://pcrbio.com/resources/tm-calculator/. For TaqMan probes choose a probe close to the 5' primer, avoiding terminal guanosine residues.

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

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

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

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara® Probe 1-Step Mix No-ROX. Add ROX as required.
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 (10 µM)	0.8-2 µL	400 nM - 1 µM	See above for optimal primer design
Reverse primer (10 µM)	0.8-2 µL	400 nM - 1 µM	
Probe (10 µM)	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	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