



PCRBIO SYSTEMS
simplifying research

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Clara® Probe 1-Step Mix Separate-ROX UNG

Product description

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

Clara® Probe 1-Step Mix is a 4x qPCR mix containing hot start Taq polymerase, dNTPs, MgCl₂, 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.

The mix also contains dUTP and UNG (uracil N-glycosylase) in the mix, to aid in preventing DNA carryover contamination.

A separate tube of 50 µM ROX additive is provided, enabling use on all real time instruments.

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 Mix No-ROX UNG	50 µM ROX Additive
600 reactions	3 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. Always keep the mix on ice during reaction setup, with handling at room temperature limited to the necessary minimum. If stored correctly, the kit will retain full activity until the indicated expiry date.

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

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

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 UNG	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://www.primer3plus.com/>). 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.

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara® Probe 1-Step Mix No-ROX UNG. Add ROX as required.
2. Always keep the UNG mix cold. Limit handling at room temperature to the necessary minimum.
3. Prepare a master mix based on the following table:

Reagent	20 µL reaction	Final concentration	Notes
4x Clara® Probe 1-Step Mix UNG	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 ₂ O	Up to 20 µL final volume		

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

Cycles	Temperature	Time	Notes
1	25 °C	2 min	UNG activity step, degrades dUTP-containing DNA
1 <i>Optional</i>	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