PCRBIOSYSTEMS
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Clara® Probe 1-Step Mix AquaPlex

Product description

Clara® Probe 1-Step Mix AquaPlex 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, even from highly dilute samples.

Clara® Probe 1-Step Mix AquaPlex 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.

The mix is formulated with a passive reference dye that has a fluorescence emission spectrum suitable for red (Cy5) channel detection, allowing use of the ROX channel for probe dyes, resulting in improved data quality.

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 AquaPlex
200 reactions	1 x 1 mL
600 reactions	3 x 1 mL
1000 reactions	5 x 1 mL
10000 reactions	1 x 50 mL

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. For 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: This product is compatible with instruments that allow use of the red (Cy5, 650 nm) channel for passive reference data collection. Use our qPCR Selection Tool to determine which instruments are suitable (https://pcrbio.com/resources/qpcr-selection-tool/).

Passive reference dye: Unlike the standard passive reference dye, ROX, only one concentration of this dye is required across all compatible instruments. Use of Cy5 or similar probe dyes can increase the background signal with this mix. Therefore, use of a mix version without passive reference dye or with ROX is recommended to mitigate this issue.

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.

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.

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara® Probe 1-Step Mix AquaPlex.
2. Prepare a master mix based on the following table:

Reagent	20 µL reaction	Final concentration	Notes
4x Clara® Probe 1-Step Mix AquaPlex	5 µL	1x	
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) and using the Cy5 channel for passive reference data collection:

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