PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

Clara™ Probe Mix Separate-ROX

Product description

Clara™ Probe Mix offers reliable probe-based qPCR detection of DNA target sequences. This 4x mix gives superior target amplification, in single or multiplex assays, even from highly dilute samples.

Clara™ Probe Mix is a perfectly balanced qPCR mix containing hot start Taq polymerase, dNTPs, and $MgCl_2$. 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. Clara™ Probe Mix Separate-ROX is supplied as Clara™ Probe Mix No-ROX along with separate 50 µM ROX additive.

We have tested this mix against standard housekeeping genes, such as g-actin and GAPDH, and evaluated this mix extensively in single and multiplex assays to secure maximum perfomance in any experimental setting.

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 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. If stored correctly the kit will retain full activity for 12 months. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month.

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: Different real-time PCR instruments require different levels of ROX passive reference. Generally, modern instruments do not need passive reference but include the option to use it for normalisation. 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 cDNA or DNA synthesised or 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. For genomic DNA, 1 µg or less is recommended. For cDNA, 100 ng or less is recommended. However, users are encouraged to attempt a dilution series for new template/primer pairs to ensure that the PCR is efficient at that template concentration.

Primer design: For efficient amplification under fast cycling conditions we recommend amplicon lengths between 80 bp and 200 bp. With all manufacturers master mixes the shorter the amplicon length the faster the reaction can be cycled. Amplicon lengths should not exceed 400 bp. Primers should have a predicted melting temperature of around 60 ˚C, using default Primer 3 settings (https://bioinfo.ut.ee/primer3/). For TaqMan® probes choose probe close to 5' primer, avoid terminal guanosine residues.

Hi-ROX instruments	Reagent volume	Final concentration	Reaction concentration
4x Clara™ Probe Mix No-ROX	1.0 mL	4x	1x
50 µM ROX Additive	40.0 µL	2 µM	500 nM

Lo-ROX instruments	Reagent volume	Final concentration	Reaction concentration
4x Clara™ Probe Mix No-ROX	1.0 mL	4x	1x
50 µM ROX Additive	4.0 µL	200 nM	50 nM

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara™ Probe Mix No-ROX, add ROX as necessary.
2. Prepare a master mix based on the following table.

Reagent	20 µL reaction	Final concentration	Notes
4x Clara™ Probe 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	See above for optimal primer design
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	
DNA Template	2-5 µL	Variable	<100 ng cDNA, <1 µg genomic DNA,
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	95 ˚C	3 minutes	Polymerase activation
50	95 ˚C 55 ˚C to 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