PCRBIOSYSTEMS
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Clara™ Probe Purple Mix No-ROX

Product description

Clara™ Probe Purple Mix offers reliable probe-based qPCR detection of DNA target sequences, combined with an inert purple dye for sample visualisation. This 4x mix gives superior target amplification, in single or multiplex assays, even from highly dilute samples.

Clara™ Probe Purple 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.

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.

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 Purple Mix No-ROX
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 -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 until the indicated expiry date. 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: This mix is intended for use on insturments with No-ROX requirement. 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.

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 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%

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.

Reaction setup

1. Before starting, thaw and briefly vortex the 4x Clara™ Probe Purple Mix
2. Prepare a master mix based on the following table.

Reagent	20 µL reaction	Final concentration	Notes
4x Clara™ Probe Purple Mix	5 µL	1x	
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