



**PCRBIO SYSTEMS**  
simplifying research

[www.pcrbio.com](http://www.pcrbio.com)

## Clara® Probe Mix Hi-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 contains hot start Taq polymerase, dNTPs, and MgCl<sub>2</sub>. It is developed to work well with the full range of probe types, including TaqMan, Scorpions and molecular beacons.

### 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 Hi-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 -20 °C. 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.

### 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](mailto: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/>).

**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 we recommend amplicon lengths between 80-200 bp and not exceeding 400 bp. Shorter amplicons allow for faster cycling. Primers should have an approximate T<sub>m</sub> 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 Mix Hi-ROX.
2. Prepare a master mix based on the following table:

| Reagent                     | 20 µL reaction           | Final concentration | Notes                               |
|-----------------------------|--------------------------|---------------------|-------------------------------------|
| 4x Clara® Probe Mix         | 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        |                                     |
| DNA Template                | 2-5 µL                   | Variable            | <100 ng cDNA, <1 µg genomic DNA     |
| PCR grade dH <sub>2</sub> O | 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             | 95 °C                            | 3 minutes     | Polymerase activation                                                   |
| 50            | 95 °C                            | 5-15 seconds  | Denaturation                                                            |
|               | 55 °C to 65 °C                   | 20-30 seconds | Anneal/Extension                                                        |
| Melt analysis | Refer to instrument instructions |               | Optional melt profile analysis, available for hybridisation probes only |