Clara ${ }^{\text {TM }}$ Probe 1-Step Mix No-ROX

## Product description

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

Clara ${ }^{\text {™ }}$ Probe 1 -Step Mix is a $4 x$ qPCR mix containing hot start Taq polymerase, dNTPs, $\mathrm{MgCl}_{2}$, an enhanced version of UltraScript ${ }^{\text {tM }}$ Reverse Transcriptase, and our RiboShield ${ }^{\text {™ }}$ 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 ${ }^{\circledR}$ and molecular beacons and can be used both for diagnostic and basic research puproses.

Our extensive optimisation makes this mix suitable for all nucleic target types. We have tested it against common RNA viruses, including SARS-CoV-2, RSV, Influenza A, and B, standard housekeeping genes, such as g-actin and GAPDH, as well as DNA targets.

## 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 | $4 \times$ Clara $^{\text {TM }}$ Probe 1 -Step Mix <br> No-ROX |
| :--- | :--- |
| 200 reactions | $1 \times 1 \mathrm{~mL}$ |
| 600 reactions | $3 \times 1 \mathrm{~mL}$ |
| 1000 reactions | $5 \times 1 \mathrm{~mL}$ |
| 10000 reactions | $1 \times 50 \mathrm{~mL}$ |

## Shipping and storage

On arrival the kit should be stored between $-30^{\circ} \mathrm{C}$ and $-15^{\circ} \mathrm{C}$. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. Avoid exposure of the stock solution to frequent temperature changes and limit handling at room temperature to the necessary minimum.

## 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 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 $5 \mu \mathrm{~L}$ volumes of sample will improve assay precision.

## Reaction setup

1. Before starting, thaw and briefly vortex the $4 x$ Clara $^{\text {TM }}$ Probe 1 -Step Mix.
2. Prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

| Reagent | $20 \mu \mathrm{~L}$ reaction | Final concentration | Notes |
| :---: | :---: | :---: | :---: |
| 4x Clara ${ }^{\text {TM }}$ Probe 1-Step Mix | $5 \mu \mathrm{~L}$ | 1x |  |
| Forward primer (0.1-1 mM) | 1-2 $\mu \mathrm{L}$ | $400 \mathrm{nM}-1 \mu \mathrm{M}$ |  |
| Reverse primer (0.1-1 mM) | 1-2 $\mu \mathrm{L}$ | $400 \mathrm{nM}-1 \mu \mathrm{M}$ |  |
| Probe (0.1-1 mM) | 0.25-1 $\mu \mathrm{L}$ | 125-500 nM |  |
| RNA or DNA Template | $2-5 \mu \mathrm{~L}$ | Variable | $<100 \mathrm{ng}$ cDNA, $<1 \mu \mathrm{~g}$ genomic DNA, $1 \mathrm{pg}-1 \mu \mathrm{~g}$ total RNA, $>0.01 \mathrm{pg}$ mRNA, 4 to $1 \times 10^{8}$ copies viral RNA |
| PCR grade $\mathrm{dH}_{2} \mathrm{O}$ | Up to $20 \mu \mathrm{~L}$ 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 Optional | $52{ }^{\circ} \mathrm{C}$ | 5-10 minutes singleplex 10-20 minutes multiplex | Reverse transcription. <br> Required only for RNA templates. |
| 1 | $95^{\circ} \mathrm{C}$ | 3 minutes | Polymerase activation and RTase inactivation |
| 40-50 | $\begin{aligned} & 95^{\circ} \mathrm{C} \\ & 55^{\circ} \mathrm{C}-65^{\circ} \mathrm{C} \end{aligned}$ | $\begin{aligned} & 5-15 \text { seconds } \\ & 20-30 \text { seconds } \end{aligned}$ | Denaturation Anneal/Extension |
| Melt analysis | Refer to instru |  | Optional melt profile analysis, available for hybridisation probes only |

