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Pre-Lyo Probe 1-Step Evaluation Kit

Product description

Pre-Lyo Probe 1-Step Evaluation Kit is a version of the Lyo-Ready Probe 1-Step Kit (PB90.14) that has been designed for evaluation purposes.

The kit includes a glycerol-free 4x qPCR mix containing hot start Taq polymerase, dNTPs, $MgCl_2$ and a blend of excipients to ensure reliable lyophilisation, without loss of activity.

UltraScript Reverse Transcriptase provided in this kit is for evaluation only. Due to its high glycerol content, it is not suitable for lyophilisation. A highly concentrated version of UltraScript® Reverse Transcriptase is available as part of the Lyo-Ready Probe 1-Step Kit.

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.

Component	600 rxns
4x Lyo-Ready Probe Mix	3 x 1 mL
20x UltraScript® RTase (with RNase inhibitor)	1 x 600 µL

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. 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. Do not store the mix once it is combined with the RTase.

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

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

Template: The kit can be used with RNA extracted by most commercial kits, provided the amount and quality of template RNA are within an acceptable range. Addition of sample as 2 to 5 µL volumes will improve assay precision. 5 µL of swab extract is recommended for diagnostic assays.

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, briefly vortex 4x Lyo-Ready Probe Mix
2. Prepare a master mix based on the following table. We also recommend setting up a no-RTase control:

Reagent	20 µL reaction	Final conc.	Notes
4x Lyo-Ready Probe Mix	5 µL	1x	
Forward primer (10 µM)	1-2 µL	400 nM-1 µM	
Reverse primer (10 µM)	1-2 µL	400 nM-1 µM	
Probe (10 µM)	0.25-1 µL	125-500 nM	
20x UltraScript® RTase	1-2 µL	1x	
RNA template	2-5 µL	Variable	4 to $1x10^8$ viral copies per reaction. See above for further template considerations.
PCR grade dH_2O	Up to 20 µL final volume		

3. Program the instrument using the following conditions, acquiring data on the appropriate channel:

Cycles	Temperature General	Temperature SARS-CoV-2 Detection	Time	Notes
1	45 ˚C to 55 ˚C	55 ˚C	5-10 minutes singleplex 10-20 minutes multiplex	Reverse transcription
1	95 ˚C	95 ˚C	3 minutes	Polymerase activation and RTase inactivation
50	95 ˚C 55 ˚C to 65 ˚C	95 ˚C 58 ˚C	15 seconds 30 seconds	Denaturation Anneal/Extension
Melt analysis	Refer to instrument instructions			Optional melt profile analysis, available for hybridisation probes only