# CelEct Virus Universal Probe One-step kit

| | |
|---|---|
| CM3711-0200 | 200 rxns |
| CM3711-0600 | 600 rxns |
| CM3711-0600 | 1000 rxns |

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## Shipping and storage instructions

**Store the kit at -20°C upon arrival.** Full enzyme activity is retained for 12 months when stored at the recommended conditions and can endure up to 30 freeze/thaw cycles. Limit direct light exposure for extended periods.

## What's in the box

| Cat no. | Pack size | 4X CelEct Virus Universal Probe One-step mix (no-ROX) | 20X CelScript Reverse | ROX Additive (50µM) |
|---|---|---|---|---|
| CM3711-0200 | 200 rxns | 1 x 1ml | 1 x 0.2ml | 1 x 0.2ml |
| CM3711-0600 | 600 rxns | 3 x 1ml | 1 x 0.6ml | 1 x 0.2ml |
| CM3711-0600 | 1000 rxns | 1 x 5ml | 1 x 1ml | 1 x 0.2ml |

## Notes

For research use only.

## Product description

The CelEct Virus Universal Probe One-step kit is developed for fast and reproducible detection of viral RNA targets using probe-based real time one-step PCR.  It can achieve sensitivity down to 4 copy inputs of RNA target and is compatible with probes based on various principles, including TaqMan®, Scorpions® and molecular beacon probes.

The 4X reaction mix allows addition of more template, increasing the sensitivity even in low reaction volumes. In addition to the advanced buffer composition, an RNase inhibitor protects viral RNA from degradation by RNases and complements the thermostable CelScript Reverse Transcriptase.

The kit is suited for multiplex assays as the antibody-mediated hot-start polymerase results in greater PCR specificity and high sensitivity, allowing accurate detection of viral RNA templates across a broad dynamic range.

The sensitivity and reproducibility make this kit ideal for high throughput detection of RNA viruses.

# Detection of SARS-CoV-2

The CelEct Virus Universal Probe One-step kit has been validated, using the Charité (Berlin, Germany) recommended primer-probe sequences (RdRp and E genes)[1], and CDC (Atlanta, USA) primer-probe sequences (N gene)[2], for the qualitative detection of SARS-CoV-2 nucleic acid. For further information please email info@celticdiagnostics.com.

[1]Diagnostic detection of 2019-nCoV by real-time RT-PCR (https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf)

[2]2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes (https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf)

## Instrument compatibility

The CelEct Virus Universal Probe One-step kit is suitable for use on all real time PCR cyclers. The Universal qPCR mix is supplied with a separate tube of 50µM ROX dye that may be used with instruments requiring ROX as a passive reference dye. Please refer to the instrument manufacturer's manual to confirm the required ROX concentration.

### ROX protocol:

Add the 50µM ROX additive directly to the 1ml tube of 4X CelEct Virus Universal Probe One-step mix and vortex thoroughly. Use immediately or store as per normal storage instructions. Refer to the directions below for guidelines to obtain the desired concentration.

*Hi-ROX instruments:*

Add 20µl of ROX additive (50µM) to 1ml of 4X CelEct Virus Universal Probe One-step mix to obtain a final concentration of 1µM. This will result in a concentration of 500nM per 20µl reaction.

*Lo-ROX instruments:*

Add 2µl of ROX additive (50µM) to 1ml of 4X CelEct Virus Universal Probe One-step mix to obtain a final concentration of 100nM. This will result in a concentration of 50nM per 20µl reaction.

## Reaction set-up

Briefly vortex the 4X CelEct Virus Universal Probe One-step mix to ensure homogeneity.

Prepare a master mix using the guidelines below. It is recommended to include a no-RTase control.

| Reagent | 20µl reaction | Final concentration |
|---|---|---|
| 4X CelEct Virus Universal Probe One-step mix | 5µl | 1X |
| Forward primer (10µM) | 1 to 2µl | 400nM to 1µM |
| Reverse primer (10µM) | 1 to 2µl | 400nM to 1µM |
| Probe (10µM) | 1µl | 125 to 500nM |
| 20X CelScript Reverse Transcriptase | 1µl | 1X |
| Template RNA | 2 to 5µl | 4 to $1x10^8$ viral copies per reaction. See template considerations. |
| PCR grade water | Up to a final volume of 20µl | |

# Recommended cycling conditions

| Description | Temperature | Time | Cycles |
|---|---|---|---|
| Reverse transcription | 45 to 55°C | 5min for singleplex<br>10min for multiplex | 1 |
| RT deactivation and polymerase activation | 95°C | 3min | 1 |
| Denaturation | 95°C | 15sec | 50 |
| Annealing and extension* | 55 to 65°C | 30sec | |
| Melt analysis (optional for hybridization probes only) – refer to the instrument manual | | | |

*Data acquisition in the relevant detection channels

# General Considerations

**Template:** Template RNA should be of acceptable quantity and quality and can be isolated using most commercially available kits/methods. It is recommended to add 2 to 5µl RNA template per reaction to improve precision. For detection of SARS-CoV-2, 5µl of swab extract is recommended.

**Primer/Probe design:** Design primers with a predicted melting temperature of around 60°C using software such as Primer 3 (http://frodo.wi.mit.edu/primer3/). In the case of TaqMan® probes, avoid terminal guanosine nucleotides and design the probe to bind in proximity to the 5' primer. The amplicon should be between 80 and 200bp in length to allow efficient amplification under fast cycling conditions, and no longer than 400bp for standard cycling conditions. Bear in mind that shorter amplicons will result in quicker cycling times.

# Troubleshooting

Non-specific amplification (presence of more than one peak during melt analysis when using hydrolysis probes) – possible actions to resolve the problem:

1. Increase the reaction annealing temperature to enhance the specificity of priming.
2. Systematically eliminate potential contamination in reaction components by replacing single components in separate PCR reactions, until the source of contamination is identified and isolated.
3. Reduce primer/probe concentration to increase specificity of priming.
4. Perform PCR set-up and PCR product analysis in separate areas.

## Troubleshooting

No PCR product visible – possible actions to resolve the problem:

1. Confirm all required components were added (in the correct volumes) during PCR reaction set-up. Ensure the relevant detection channel is selected in the instrument software for successful signal acquisition.
2. Ensure components were stored at the recommended storage conditions listed above. Test each component in separate reactions to eliminate potential defective reagent.
3. Decrease the amount of DNA added to the reaction to reduce PCR inhibitors that may be present in samples.
4. Decrease the annealing temperature to reduce the primer specificity.
5. Increase the number of cycles to allow additional exponential amplification.

# Troubleshooting

**Amplification in the no-RT control** – possible actions to resolve problem:

1. Eliminate the presence of genomic DNA that may amplify without the need of reverse transcription (e.g. DNase treatment)
2. Systematically eliminate potential contamination in reaction components by replacing single components in separate PCR reactions, until the source of contamination is identified and isolated.

# Technical support

For technical support please e-mail

info@celticmolecular.com