CelProbe Universal qPCR mix (2x)

CM3311-0200	200 rxns
CM3311-0500	500 rxns
CM3311-1000	1000 rxns

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Shipping and storage instructions

Store the kit at -20°C upon arrival. This product is shipped on ice blocks and can be kept at 4°C for 4 weeks without affecting the product performance. 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	2x CelProbe Universal qPCR mix	ROX Additive (50µM)
CM3311-0200	200 rxns	2 x 1ml	1 x 0.2ml
CM3311-0500	500 rxns	5 x 1ml	1 x 0.2ml
CM3311-1000	1000 rxns	10 x 1ml	2 x 0.2ml

Notes

For research use only.

Product description

CelProbe Universal qPCR mix combines an antibody-mediated hot-start polymerase with an advanced buffer system to achieve superior PCR sensitivity and greater PCR specificity by limiting the formation of primer dimers. This mix performs well with AT- and GC-rich templates and requires minimal optimization. The CelProbe Universal qPCR mix can be used with probes based on various probe principles, including TaqMan®, Scorpions® and molecular beacon probes.

Instrument compatibility

CelProbe Universal qPCR mix 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 2x CelProbe Universal qPCR 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 2x CelProbe Universal qPCR 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 2x CelProbe Universal qPCR 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 2x CelProbe Universal qPCR mix to ensure homogeneity.

Prepare a master mix using the guidelines below.

Reagent	20µl reaction	Final concentration
2x CelProbe Universal qPCR mix	10µl	1x
Forward primer (10µM)	0.8µl	400nM
Reverse primer (10µM)	0.8µl	400nM
Probe (10µM)	0.4µl	200nM
Template DNA	Variable	See template considerations
PCR grade water	Up to a final volume of 20µl	

Recommended cycling conditions

Description	Temperature	Time	Cycles
Enzyme activation and initial denaturation. Use 2min for cDNA and 3min for genomic DNA	95°C	2 to 3min	1
Denaturation	95°C	5sec	40
Annealing and extension, maximum 30sec, minimum 60°C*	60 to 65°C	20 to 30sec	
Melt analysis (optional for hydrolysis probes only) – refer to the instrument manual			

*Data acquisition in the relevant detection channels

General Considerations

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.

Template: Add 1µg or less of high-quality genomic DNA per 20µl reaction or up to 100ng cDNA per 20µl reaction. Perform PCR with a template dilution series to determine the optimal amount of template to use with new primer pairs for maximum amplification efficiency.

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 the primer/probe concentration to increase the 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 primer specificity.
5. Increase the number of cycles to allow for additional exponential amplification.

Technical support

For technical support please e-mail

info@celticmolecular.com