CelTaq DNA Polymerase (2x)

CM2211-0500 500 units

CM2211-1000 1000 units

CELTIC MOLECULAR

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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.	CM2211-0500	CM2211-1000
Pack size	500 units	1000 units
CelTaq DNA polymerase, 5U/µl	1 x 0.1ml	2 x 0.1ml
CelTaq buffer, 5x	4 x 1ml	8 x 1ml

Notes

For research use only.

Product description

CelTaq DNA Polymerase is a robust PCR enzyme, suited for routine PCR applications including genotyping, high throughput screening, PCR from crude extracts, library construction and TA cloning.

Improved buffer chemistry allows for consistent and efficient amplification under standard and fast cycling conditions, producing high amplicon yields with superior specificity (even with complex DNA targets rich in GC or TA content).

Properties

CelTaq DNA polymerase is the ideal choice for routine PCR applications, with the advanced buffer system that enables efficient, consistent amplification of complex (GC-rich and AT-rich) templates.

Error rate: 1 in 2.0 x 105 incorporated nucleotides

Proofreading ability: 5′-3′ exonuclease activities, but no 3′-5′ exonuclease (proofreading) activity

Amplicon length: up to 6 kb

Amplicon 3′ end: A-tailed

Reaction set-up

Prepare a master mix using the guidelines below.

Reagent	25µl reaction	Final concentration
CelTaq buffer, 5x	5µl	1x
Forward primer (10µM)	1µl	400nM
Reverse primer (10µM)	1µl	400nM
Template DNA	Variable	See template considerations
CelTaq DNA polymerase, 5U/µl	0.2 – 0.5µl	
PCR grade water	Up to a final volume of 25µl	

Recommended cycling conditions

Description	Temperature	Time	Cycles
Initial denaturation	95°C	1min	1
Denaturation	95°C	15sec	40
Annealing	55 to 65°C	15sec	
Extension (15sec per kb)	72°C	1 to 90sec	

General Considerations

Template: Add 2.5 to 250ng of eukaryotic DNA per 25µl reaction or up to 50ng cDNA per 25µl reaction.

CelTaq buffer, 5x: The 5x buffer comprises of 5mM dNTPs, 15mM MgCl2, stabilizers and enhancers. Supplementing the 5x reaction buffer with additional components is not recommended as it has been optimized for maximum PCR success.

Primers: Add primers to reach a final concentration of between 0.2 and 0.6µM per reaction. Design primers with a predicted melting temperature of around 60°C using software such as Primer 3 (http://frodo.wi.mit.edu/primer3/).

Annealing: Determine the optimal annealing temperature via a temperature gradient experiment. Alternatively, start with an annealing temperature of 55°C, increasing by 2°C increments until non-specific amplification is eliminated.

Extension: Suggested elongation time is dependent on the complexity of the template and length of the amplicon. For eukaryotic genomic DNA amplicons between 1 and 6kb in length, an extension time of 15 seconds per kilobase is recommended. 1 second elongation is adequate for shorter amplicons. The optimal temperature for amplicon extension is 72°C.

Troubleshooting

Non-specific amplification – possible actions to resolve the problem:

1. Ensure PCR reactions are set up on ice to limit polymerase activity at room temperature.
2. Increase annealing temperature to enhance the specificity of priming.
3. Systematically eliminate potential contamination in reaction components by replacing single components in separate PCR reactions, until the source of contamination is identified and isolated.
4. Reduce primer concentration to increase the specificity of priming.
5. Decrease extension time to eliminate non-specific amplification of larger amplicons.
6. Decrease the number of cycles.
7. 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 all required components (DNA safe stain and loading dye) were added prior to gel electrophoresis analysis.
2. Ensure components were stored at the recommended storage conditions listed above. Test each component in separate reactions to eliminate potential defective reagent.
3. Increase initial denaturation time to a maximum of 3 minutes to allow complete denaturation of complex DNA templates (such as eukaryotic genomic DNA).
4. Decrease the amount of DNA added to the reaction to reduce PCR inhibitors that may be present in samples.
5. Decrease the annealing temperature to reduce primer specificity.
6. Increase the extension time if amplifying a long target.
7. Increase the number of cycles to allow for additional exponential amplification.

Technical support

For technical support please e-mail

info@celticmolecular.com