CelTaq HS DNA Polymerase (2x)

CM2311-0250 250 units

CM2311-1000 1000 units

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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.	CM2311-0250	CM2311-1000
Pack size	250 units	1000 units
CelTaq HS DNA polymerase, 5U/µl	1 x 0.05ml	4 x 0.05ml
5x CelTaq HS buffer	2 x 1ml	8 x 1ml

Notes

For research use only.

Product description

CelTaq HS DNA polymerase utilizes an antibody-mediated hot start that results in greater specificity and high amplicon yields from even low copy number templates. The advanced buffer chemistry (containing dNTPs, MgCl2, stabilizers and enhancers) allows for superior PCR performance on difficult templates rich in GC/AT content (like mammalian genomic DNA). The CelTaq HS buffer is inhibitor tolerant, enabling amplification directly from blood/urine or bacterial cultures/colonies.

This robust enzyme is suitable for routine PCR applications, as well as multiplexing, genotyping, library screening and high-throughput experiments that require lengthy reaction set-up times. CelTaq HS DNA polymerase retains efficiency under standard and fast cycling conditions with consistent high yield and specificity.

Properties

CelTaq HS DNA polymerase can be used for routine PCR applications and is ideal for PCR with extended reaction set-up times, multiplexing and direct-PCR from bacterial cells, blood or urine. Increased sensitivity and specificity mediated by advanced hot-start technology result in superior performance on difficult templates (high in CG or TA content) or crude samples containing PCR inhibitors.

Error rate: 1 in 2.0 x 10^5 incorporated nucleotides

Enzyme modification: antibody mediated-hot start

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
5x CelTaq HS buffer	5µl	1x
Forward primer (10µM)	1µl	400nM
Reverse primer (10µM)	1µl	400nM
Template DNA	Variable	See template considerations
CelTaq HS 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
Enzyme activation and initial denaturation. Increase time to 10min for colony PCR	95°C	1 to 2min	1
Denaturation	95°C	15sec	40
Annealing	55 to 65°C	15sec	
Extension (15sec per kb). Increase time to 90sec for multiplex PCR	72°C	1 to 90sec	

General Considerations 1

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

CelTaq HS buffer, 5x: The 5x buffer comprises of 5mM dNTPs, 15mM $MgCl_2$, 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.

General Considerations 2

Colony PCR: Pick a single colony from a culture plate using a sterile tip and resuspend in the 25µl PCR reaction. Alternatively, add 5ul of an overnight culture directly to the PCR reaction. Increase the initial denaturation time to 10min to facilitate bacterial cell membrane rupture.

Multiplex PCR: It is recommended to use standard (not fast) cycling conditions for multiplex PCR. Perform a temperature gradient experiment to select the most efficient annealing temperature between 55 and 65°C. Use 90sec for initial cycling extension. This may be further increased during additional optimization.

Direct PCR: PCR may be performed without DNA isolation by adding 1µl blood/urine per 25µl PCR reaction.

Troubleshooting

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

1. Increase 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 concentration to increase the specificity of priming.
4. Decrease extension time to eliminate non-specific amplification of larger amplicons.
5. Decrease the number of amplification cycles.
6. 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 the potential defective reagent.
3. Increase the 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