

FideliTaq™ DNA Polymerase
Tested User Friendly™
Product Number 71180
Brief Protocol



FideliTaq™ DNA Polymerase combines high-quality USB recombinant Taq DNA Polymerase with a thermostable, proofreading polymerase. This enzyme mixture increases amplification fidelity approximately 3-6 times over Taq DNA Polymerase alone and allows for amplification of longer product sizes as well.

Protocol

This standard protocol applies to a single reaction. Master mixes for multiple reactions can be made by increasing the volumes of reaction components proportionally.

1. Thaw frozen reagents at room temperature. Mix thoroughly and then place on ice with FideliTaq DNA Polymerase.
2. Assemble reaction tubes on ice whenever possible to avoid premature, non-specific polymerase activity.
3. This table shows recommended component volumes:

Components	Vol. for 50 µl reaction	Final Concentration
10X PCR Buffer	5.0 µl	1X
10mM dNTP Mixture	1.0 µl	0.2mM each dNTP
10µM Forward Primer	0.5-5.0 µl	0.1-1.0µM†
10µM Reverse Primer	0.5-5.0 µl	0.1-1.0µM†
Template DNA	≥ 1 µl	as needed, < 500 ng
FideliTaq DNA Polymerase, 5 units/µl	0.25 µl	0.025 units/µl
Water, PCR-Qualified	up to 50 µl	NA

† Optimal primer concentration is 0.2µM or 10 pmol each primer per reaction.

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.



5. *Optional*—Overlay reactions with one-half volume PCR-grade mineral oil (PN 71600) when not using heated lid on thermal cycler.
6. This table shows recommended cycling conditions:

Cycle Step	Temperature	Time
Initial Denature	94-95°C	1-2 minutes
Denature	94-95°C	30 seconds
Anneal*	55°C	30 seconds
Extend**	68°C	1-2 minutes
Repeat previous three cycles as necessary, generally 25-35 times.***		
Final Extend	68°C	5 minutes
Final Soak	4-10°C	as necessary

* Initially, the annealing temperature should be 5°C below the calculated T_m of the primers. If non-specific products are produced, increase the annealing temperature in 1-2°C increments.

** Use an extension temperature of 72°C for products ≤ 2 kb and 68°C for products > 2 kb. Extension time should be 1 minute/kb for expected products < 5 kb and 2 minutes/kb for products ≥ 5 kb, up to a maximum of about 30 minutes.

*** 45 cycles may be required for low-copy targets.

7. Analyze sample (typically 1 to 10 µl aliquots) by agarose gel electrophoresis. Visualize PCR product in gel with DNA intercalating dyes and a UV transilluminator or fluorescence imager.

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