TaqDog TUFF Hot Start DNA Polymerase

Products

CAT. #	Description	Volume
TFTD010	TaqDog TUFF Hot Start DNA Polymerase with 10X TUFF Reaction Buffer (no dNTP), 1,000 U	1,000 Units (200 µL)
TFTD050	TaqDog TUFF Hot Start DNA Polymerase with 10X TUFF Reaction Buffer (no dNTP), 5,000 U	5,000 Units (1.0 mL)

Product Information

TaqDog TUFF Hot Start DNA polymerase is a robust next generation DNA polymerase with increased processivity and yield relative to Taq DNA polymerase. Cycling programs using this enzyme can have shorter extension times and fewer cycles to produce the same level of amplification. This polymerase also boasts significantly increased resistance to many types of PCR inhibitors, chemical denaturants, as well as increased thermo-stability (minimal loss of activity after 1 hour at 95°C). The added robustness allows for use of template DNA that is of lower quality and simpler DNA purification protocols. The **TaqDog TUFF Hot Start DNA polymerase** incorporates an aptamer-based hot start technology that provides a reversible mode of inhibition that inhibits the amplification of primer-dimer formation and non-specific products, allowing for reaction setup at room temperature.

TaqDog TUFF Hot Start DNA polymerase has 5’ – 3’ polymerase activity, lacks 3’–5’ exonuclease activity, and produces amplicons with 3’-dA-tail for cloning into TA vectors. 5'-3' exonuclease activity is removed from TaqDog TUFF and as a result this enzyme has increased thermostability and resistance to protein denaturing conditions. It is not compatible with assays that require this function, such a TaqMan™. TaqDog TUFF is ideal for Molecular Beacons™, Sybr Green™ melt curve analysis, and challenging PCR reactions such as direct-from-tissue PCR or colony PCR.

Extension: 5,000 base-pair extension has been confirmed

Speed: 3 – 4 Kbp per minute

Unit Definition: One unit of TaqDog TUFF Hot Start DNA polymerase incorporates 10 nmol of deoxyribonucleotide into acid-perceptible material in 30 minutes at 74 C.

Product Components

	5,000 U	1,000 U
TaqDog TUFF Hot Start DNA Polymerase	1 x 1 ml	1 x 200 µl
10X TUFF Reaction Buffer	1 x 50 ml	4 x 1.5 ml
50 mM $MgSO_4$	1 x 10 ml	2 x 1.5 ml
GC Extender	1 x 10 ml	1 x 1.5 ml

10X TUFF Reaction Buffer: 80 mM Tris-HCl, pH 8.5, 0.8 mg/ml BSA, 82.5 mM KCl, 82.5 mM NH_4OAc (ammonium acetate), 0.8 % Triton X-100.

All components are to be stored at - 20 °C

Protocol

1. Add following components to a PCR tube on ice:

Note: DNA amount, primer amount, Taq Polymerase units and $MgSO_4$ concentration should be optimized.

Component	Volume	Final Concentration
Template DNA (up to 500 ng)	up to 10 µl	–
10 µM Forward Primer	0.5 µl – 2.5 µl	100 nM – 500 nM
10 µM Reverse Primer	0.5 µl – 2.5 µl	100 nM – 500 nM
10X TUFF Reaction Buffer	5 µl	1 X
50 mM $MgSO_4$	1.5 µl – 3 µl	1.5 mM – 3 mM
10 mM dNTP mix	1 µl	0.2 mM
TaqDog TUFF Hot Start DNA Polymerase	0.2 µl – 1 µl	1 U – 5 U
GC-Extender (Optional)*	5 µl – 15 µl	–
Nuclease-free H_2O	up to 50 µl	–

*GC-Extender is intended for difficult-to-amplify templates (e.g. GC content > 60 % or for templates longer than 2.5 Kbp). Volume of GC-Extender required (5 µl – 15 µl) should be optimized for each template and primer combination.

2. Mix contents (either through pipetting multiple times or briefly vortexing).

3. Centrifuge briefly and proceed to PCR amplification steps.

4. PCR amplification:

Note: Number of cycles, annealing temperature and extension should be optimized for each template and primer set.

Stage	Step	Temperature (°C)	Time	Cycles
1	Denature*	98	10 sec - 30 sec	20-30
	Anneal	55 - 60	10 sec - 30 sec	
	Extend	72	1 kbp / 15 sec	
2	Final extension step	72	3 min	1
3	Holding step	4	∞	1

**The aptamer-based hot start technology does not require an additional high-temperature incubation step for activation, as is common with antibody-based methods.*

5. Product is ready for downstream analysis. For long-term storage store at -20C.

END OF PROTOCOL