DIALAT Ltd.

Data sheet
This data sheet provided for information only

StormTaq DNA POLYMERASE

RESEARCH USE ONLY

Recombinant StormTaq DNA polymerase

(Deoxynucleosidetriphosphate: DNA Deoxynucleosidyltransferase E.C. 2.7.7.7.)

Cat.No.	Pack	Conc.
DST-100	**100 Units**	**2,5 U/μl**
DST-500	**500 Units**	**2,5 U/μl**

Stability:
Shelf life 24 months if store at -20°C

UNIT DEFINITION
One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 74°C under assay conditions.

STORAGE AND DILUTION BUFFER:
20mM Tris-HCL (pH 8.0);100mM KCL; 0.1mM EDTA; 1mM DTT; 50% glycerol,0.5% Tween-20

AMPLIFICATION BUFFERS:
StormTaq polymerase supplied with the 2,5X complete buffer with the Mg2+ concentration of 3.75mM
Recommended Mg^{2+} concentration 1,5-2.5mM

SOURCE:
Thermus Auqaticus strain and genetic modifications

DESCRIPTION
StormTaq DNA Polymerase is a "next generation" chimeric enzyme, genetically constructed from point-mutated version of ***N-terminally truncated*** Thermus ***aquatiqus*** DNA polymerase...
The mutations, which inserted into truncated Taq DNA polymerase, allow ***StormTaq*** to works with the partly cleaned DNA samples contained PCR inhibitors (especially from the blood samples).
Unique properties of ***StormTaq*** helps to overcome the problems with the amplification of complex DNA templates in the presence of inhibitors (amplification from the whole blood up to 10% of reaction volume)
The enzyme catalyses polymerization of nucleotides into duplex DNA in the 5'->3' direction in presence of Mg^{2+} ions as robust Taq Polymerase. The enzyme lacks a 5'->3' exonuclease activity in comparison to its precursor, and can't be used in TaqMan probe PCR
StormTaq is more heat-stable and possessive than regular Taq polymerase. This feature allows optimizing reaction conditions easier, than with other polymerases.

ASSOCIATED ACTIVITIES:
Endonuclease/nickase and exonuclease activities were not detectable in QC tests.

APPLICATIONS:
- Routine PCR
- Primer extension
- "in blood" PCR (up to 15% of blood)
- SNP detection

STORAGE CONDITIONS :
Store ***StormTaq*** DNA Polymerase at -20°C for long term storage

SHIPPING CONDITIONS:
Should be shipped at ambient temperature
For long distance shipments preferably in **Blue Ice**

DIALAT Ltd., 117 149, Moscow, Russia, Sympheropolsky blv.8, Tel. +499 613 2052, Fax. +499 613 4818
e-mail: info@dialat.ru web: www.dialat.ru

General amplification protocol with StormTaq DNA Polymerase

PCR amplification conditions with StormTaq polymerase very similar to the ones with the KlenTaq polymerase, StormTaq polymerase is better working at the elevated temperatures of template DNA denaturation and primers annealing.
General recommendation – set up the PCR reaction on ice (+4°C), since StormTaq has no in strict "hot-start", to avoid possible miss-priming and non-specific amplification.
To set up your PCR it is better to prepare Mastermix first for the calculated+2 PCR reaction, according the following proportions.

Mix the following components on ice:

Component	50μL reaction	25μL reaction	Final 1X concentrations
PCR grade water	to 50 μL	to 25 μL	
2.5x Uni Buffer-1500*	20 μL	10 μL	1X
2,5 mM MIX dNTPs	4 μL	2 μL	0.2 mM each
Primers			0.3-0.5 μM each
DNA template**	optional	optional	from 20 ng
StormTaq polymerase (2,5 U/μl)	0.5-1 μL	0.25-0.5 μL	

***-** 2,5X Uni Buffer-1500 for StormTaq initially contains 3.75mM $MgCl_2$ (1.5mM -1X).In some cases it's necessary to optimize the Mg concentration to find the best PCR conditions for the each primers pair and DNA template. Usually the most effective $MgCl_2$ concentration interval is 1,5-2,5mM.
Recommended PCR reaction final volume – 25-50 μl.
** - DNA template could be substituted with whole blood, (fresh) or EDTA-stabilized (up to 10% of the final PCR reaction volume).
In case of usage of whole blood sample in PCR it' s recommended to increase the $MgCl_2$ concentration to 2.5-3.0mM in 1X concentration.

Amplification protocol

Cycle step	3-stage PCR (DNA template)		3-stage PCR (blood sample)		Cycles
	T°C	Time	T°C	Time	
Initial denaturation	**98°C**	**2-3 min**	**98°C**	**5 min**	**1**
Denaturation	**94-95°C**	**5-15 sec**	**98°C**	**5-15 sec**	
Annealing	**60-72***	**10-15 sec.**	**60-72***	**10-15 sec**	**35-40**
Elongation	**72°C**	**15-30 sec**	**72°C**	**15-30 sec****	
Final elongation	**72°C**	**1-3 min**	**72°C**	**1-3 min**	**1**
	4°C	**hold**	**4°C**	**hold**	

***-** Optimal Tm, is recommended as a lowest melting temperature of one of the primers, for the standard oligonucleotides <22 nt.
For **StormTaq** polymerase correction of the annealing Temp, in the range +3-6°C needed, in comparison to the optimal PCR condition with the regular Taq-based enzymes; because of containing of the additives in 2,5X Reaction Buffer lowering denaturation Temp.

1. Denaturation:

-1) Initial denaturation for 5 min at 98°C is necessary only for blood cells lysis;
-2) for most applications, including "direct-blood" 5 sec at 98°C is enough for denaturation of the sample in during PCR run

NOTE: Do not use lower T denaturation, then 98°C; it can cause problems in PCR (nonspecific amplification, poor yield of PCR product, etc.)

2. Annealing/Extension:

For **HF-Fuzz** DNA polymerase "Annealing" and "Extension" steps could be combined if:
-Tm of both primers are not differs dramatically (<3°C);
-Tm of the primers are >65°C (optimal Tm for the primers lays between 65-70°C)
If primers Tm is about 60-61°C for both primers ones can apply simple formula to determine starting Ta/e point - (Tm of the lower primer +72°C)/2. For most applications, it works fine.
To determine a better Ta/e run gradient amplification.
To avoid nonspecific band formation/smearing during amplification not exceed extension time of 30 seconds and use the highest ramp rate of amplificator (the ramp rate >4-5°C preferable)

**For non-complex DNA templates (plasmid DNA, phage DNA, BAC clone) extension time could be reduced up to 15 sec/Kb or less, up to 5 sec, depending on the length of amplicon.
For complex DNA templates (human DNA) strongly recommended to apply Extension time as 30 sec/Kb for templates > 1, 5 Kb.

DIALAT Ltd., 117 149, Moscow, Russia, Sympheropolsky blv.8, Tel. +499 613 2052, Fax. +499 613 4818
e-mail: info@dialat.ru web: www.dialat.ru