**DIALAT Ltd.**

**Data sheet**
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# HS-Fuzz DNA POLYMERASE

**RESEARCH USE ONLY**

## Recombinant HS-Fuzz DNA polymerase

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

| Cat.No. | Pack | Conc. |
|---|---|---|
| **DHSF-100** | **100 Units** | **2 U/μl** |
| **DHSF-500** | **500 Units** | **2 U/μl** |

**Stability:**
Shelf life 18 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.

### ASSOCIATED ACTIVITIES:

Endonuclease and exonuclease activities were not detectible after 2 and 1 hours incubation, respectively, of 1 μg lambda DNA and 0.22 μg of EcoR I digested lambda DNA, respectively, at 72°C in the presence of 15-20 units of **UlrtaSmarTaq** DNA polymerase.

### STORAGE AND DILUTION BUFFER:

20mM Tris-HCL (pH 8.0);100mM KCL;0.1mM EDTA; 50% glycerol,1% Tween-20, Stabilizer

**OR (Glycerol Free)**

20mM Tris-HCL (pH 8.0);100mM KCL;0.1mM EDTA;1% Tween-20, Stabilizer

### AMPLIFICATION BUFFERS:

**HS-Fuzz** DNA Polymerase is supplied with **2.5x Uni Buffer-1500**, containing 3.75 mM

### STORAGE CONDITIONS :

Store ***HS-Fuzz*** 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**

### SOURCE:

***E.coli*** strain expressing the cloned **HS-Fuzz** DNA Polymerase gene. **Anti-Pfu** mouse monoclonal antibodies

### DESCRIPTION

**HS-Fuzz** DNA Polymerase is a unique artificial enzyme created on the basis of intellectual protein design planning by genetic engineering technique.
HS-Fuzz emzymatic activity at ambient temperature is blocked by highly specific monoclonal antibodies against HF-Fuzz polymerase. Antibodies allows to switch enzymatic activity during first denaturation step of PCR run, but keeps HF-Fuzz inactive during PCR reaction setup.
This feature results in improovement of specificity of PCR and increasing of yeild of PCR product, preventing miss priming and PCR artifacts formation – primer-dimers.
The enzyme possess **high fidelity** feature. The processivity of the enzyme is very high, so the combination of processivity with fidelity results in dramatically increased yield of PCR products, very high sensitivity of PCR tests, ability to amplify "difficult" templates.This dramatic increase in processivity results not only in shorter extension times, but in more robust amplification and the ability to amplify long templates really fast.
**HS-Fuzz DNA** Polymerase possesses the 5'->3' DNA polymerase activity, 3'->5' exonuclease activity and temperature-depended strand-displacement activity and generates blunt ends in the amplification products.

**HS-Fuzz** Provides:
**- Superior fidelity - 50x improvement compared to Taq polymerase;**
**- Excellent performance across a wide range of "difficult" templates;**
**- Long range amplification of complex targets -> 10 kb from genomic DNA;**
**- High speed - reduce reaction times.**
**- dUTP poisoning resistance**
**- Resistance to blood containing DNA samples (up to 20% of blood)**

### APPLICATIONS:

- Hi-Fi PCR
- Multiplex amplification
- High sensitivity applications
- Low-copy number PCR
- Complex DNA template PCR
- "Direct-blood" PCR
- "Anti-contamination" PCR
- "fast" PCR

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# General Protocol for amplification with HS-Fuzz DNA polymerase

The optimal reaction conditions for **HS-Fuzz** DNA polymerase differs from PCR protocols for standard (Taq-like) DNA polymerases. PCR conditions for **HS-Fuzz DNA** Polymerase is similar in PCR conditions to **Phusion (ThermoScientific) ,Q5 polymerase (NEB)** or **iProof (Bio-Rad)** DNA polymerase, *e.g.* **HS-Fuzz** polymerase works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up even at room temperature, because of blocking of enzymatic activity by **specific monoclonal antibodies** at PCR setup conditions (usually RT).

**HS-Fuzz** activates without pre-heating step, during "**Initial Denaturation**" PCR step. There is no need to enter additional heating step during PCR run.

**Add and mix the following component on ice:**

| Component | 50μL reactions | 25μL reactions | Final concentration |
|---|---|---|---|
| PCR grade Water | Up to 50 μL | Up 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 |
| Template DNA | optionally | optionally | |
| **HS-Fuzz** (2 U/μl)* | 0,5-0,25 μL | 0.25-0,125 μL | 0.02U/μL |

**We recommend to use 50μl reaction for the PCR with HS-Fuzz polymerase**

## Reaction Components

**1. HS-Fuzz Polymerase**

An optimal amount of enzyme in 50μl reaction is 1U. In some cases it could be reduced up to 0.5U per reaction, depending on the length and complicity of amplified DNA sequence. For non-complex amplicons with the length less than 500bp and GC-content <60% amount of HS-Fuzz could be decreased up to 0.5-1.25U per 50μlreaction.

**2. Buffer**

2.5 X Uni HS-Fuzz buffers provides very high reproducibility across the wide range of amplification conditions, including "fast-PCR" (reduced time of PCR reaction). 2,5X Uni Buffer contains 1.5mM $Mg^2$+, as the final concentration. In some cases we recommend to optimize Mg concentration in the range 1.5-4.5mM

**3. DNTP's**

For most of applications 200μM of each of dNTP's as final concentration is an optimal. It's not necessary to optimize dNTP's concentration. dUTP or other dUTP derivatives should be used replacing TTP in PCR reaction for "anti-contamination" PCR.

**4. Primers**

Usually 10-20pmol of each specific primer in reaction is enough to get good PCR result. If you are using 2-step PCR with the whole blood as a template, it's better to use >= 20pmol of each primer.

**5. PCR Additives**

**HS-Fuzz** polymerase is compatible with the most of commonly used PCR additives for enhancing of high GC-content DNA templates (glycerol, betaine, DMSO and other).If one will use any additives; take into account the changes of Tm of primers and DNA to correct annealing temperature.

## Cycling Protocol:

| | 2-step amplification | | 3-step amplification | | |
|---|---|---|---|---|---|
| **Cycle step** | **T°C** | **Time** | **T°C** | **Time** | **Cycles** |
| **Initial Denaturation** | **98°C** | **1-5 min** | **98°C** | **1-5min** | **1** |
| **Denaturation** | **98°C** | **2-10 S** | **98°C** | **2-10 S** | |
| **Annealing** | **-** | **-** | **58-72*** | **10-30 S** | **25-35** |
| **Extension** | **66-72°C** | **15-30 S/Kb** | **72°C** | **15-30 S/Kb**** | |
| **Final extension** | **72°C** | **1-3 min** | **72°C** | **1-3 min** | **1** |
| | **4°C** | **hold** | **4°C** | **hold** | |

*Optimal Tm for the primer pair recommended as Tm of the lower primer, for the standard oligos <20nt.

For **HS-Fuzz** polymerase Tm of the primers should be corrected, as +3-10°C, comparing with "regular" Taq-based enzymes PCR conditions.

**NOTE: If whole blood is using in PCR reaction as a template, it's highly recommended to hold primers concentration above 15 pmol (preferable 20-25 pmol) and to titrate Mg concentration from 2mM (1X) up to 3, 5-4mM, depending on the volume of blood in PCR.**

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**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: Don't 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 **HS-Fuzz** polymerase "Annealing" and "Extension" steps should 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.

For complex DNA templates (human DNA) in amplification of the fragments longer than 2Kb strongly recommended to apply Extension time as 30 sec/Kb

For the DNA templates with GC content less 65% and up to 500bp length it's quite enough to set Extension time as 15 sec per cycle

**3. Post-PCR amplicon detection:**

- If you are using whole blood as a template, after amplification spine-down blood cells debris to avoid its application to the gel for DNA detection.

**Note: Don't use excess of the blood (>10%), because the post-PCR debris volume of blood cells is very high, and not allow to run more than 1-2 gels.**