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

**RESEARCH USE ONLY**

## Recombinant HF-Fuzz DNA polymerase

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

| Cat.No. | Pack | Conc. |
|---|---|---|
| **DHFF-100** | **100 Units** | **2 U/μl** |
| **DHFF-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 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM $MgCl_2$ , 1 mM β-mercaptoethanol, 200 μM each dNTP, and 10 μg activated calf thymus DNA in 50 μl.

**STORAGE AND DILUTION BUFFER:**
20mM Tris-HCl, pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% Glycerol,0.5% Tween 20 and stabilizers.

**AMPLIFICATION BUFFERS:**

HF-FUZZ DNA Polymerase is supplied with **2.5x Uni** HF-Fuzz Buffer, containing 3.75 mM $MgCl_2$ in the provided 2. 5x concentration.

**SOURCE:**
***E.coli*** strain expressing the cloned **HF-Fuzz** DNA Polymerase gene.

**DESCRIPTION**

**HF-Fuzz** DNA Polymerase is a unique artificial enzyme created on the basis of intellectual protein design planning by genetic engineering technique. 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 also in more robust amplification and the ability to amplify long templates really fast.

**HF-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.

**HF-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)**

**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 **HF-Fuzz** DNA polymerase.

**APPLICATIONS:**
- High Fidelity (Hi-Fi) PCR
- Cloning
- "Hi–Fi" LD PCR
- "anti-contamination" PCR
- "direct-blood" PCR
- GC-rich templates amplification
- " fast-PCR"

**STORAGE CONDITIONS** :
Store **HF-Fuzz** DNA Polymerase at -20°C

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

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# General Protocol for amplification with HF-Fuzz DNA polymerase

The optimal reaction conditions for **HF-Fuzz** DNA Polymerase may differ from PCR protocols for standard (Taq-like) DNA polymerases. PCR conditions for HF-Fuzz DNA Polymerase is similar in PCR conditions to "Phusion-like" DNA polymerases, *e.g.* **HF-Fuzz** polymerase works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified.

**Note! It is critical that the HF-Fuzz DNA polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.**

**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 HF-Fuzz Buffer* | 20 μL | 10 μL | 1X |
| 10 mM MIX dNTPs | 1 μL | 0.5 μL | 0.2 mM each |
| Primers | | | 0.3-0.5 μM each |
| Template DNA | optionally | optionally | |
| **HF-Fuzz** polymerase (2 U/μl) * | 0,5-0,25 μL | 0.25-0,125 μL | 0.02U/μL |

* 2,5X Uni HF-Fuzz Buffer contains 1.5mM Mg2+, as the final concentration.
In some cases, we recommend to optimize Mg concentration in the range 1.5-3.5mM
We recommend using 50μl reaction for the PCR with HF-Fuzz polymerase

## Reaction Components

**1. HF-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 **HF-Fuzz** could be decreased up to 0.5-1.25U per 50μlreaction.

**2. Buffer**
**2.5X Uni 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**
**HF-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** | **5-10 S** | |
| **Annealing** | **-** | **-** | **55-72*** | **10-30 S** | **25-35** |
| **Extension** | **68-72°C** | **15-30 S/Kb** | **72°C** | **15-30 S/Kb**** | |
| **Final extension** | **72°C** | **1-2 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 HF-Fuzz polymerase Tm of the primers should be corrected, as +3-5°C, comparing with Taq-based PCR

**NOTE**: **If you are using whole blood as template, it is crucial to use primers concentration not less than 20pmol of each per reaction. Mg concentration should be increased up to 3,5mM (1X) or higher when blood, stabilized by heparin/citrate or EDTA is using as DNA template**.

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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: 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 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 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.

**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: Do not 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.**

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