

Data sheet

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2,5X HFDBMasMIX-3510**RESEARCH USE ONLY****Ready-to-use "direct-blood" amplification MasterMix**

Cat. No.	Pack	Conc.
MMDB-100	100 rnx	2,5 X
MMDB-500	500 rnx	2,5 X

Stability:

2,5X HFDBMasMix stable for 24 months at -20°C, **or** for 6 months at +4°C storage without freezing.

CONTENT:

1X: HF-Fuzz Polymerase
0.2mM each of dNTP's
3,5 mM MgCL₂
 Reaction buffer components
 Stabilizer/enhancer

DESCRIPTION

2,5X HFDBMasMix is a ready-to-use premix of all components for amplification of target DNA directly from whole blood samples, without DNA extraction contains **stabilizer/ enhancer**, which improves thermostabilization of enzyme during PCR amplification and storage.

2,5X HFDBMasMix contains **HF-Fuzz** DNA Polymerase - 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.

2,5X HFDBMasMix provides:

- **Amplification of target DNA from blood samples or blood stains without DNA extraction (up to 15% of blood in reaction);**
- **Excellent performance across a wide range of "difficult" templates;**
- **Amplification from fresh or stabilized blood samples – blood stabilized by heparin, citrate or EDTA;**
- **High speed - reduce reaction times (only 1-1,5 sec elongation time per 100bp)**

Just place it into the tube/plate adds primers and blood sample or blood sample spotted on paper, mix all components and run PCR.

After PCR reaction running, mix 5-10µl of reaction mixture with appropriate volume of "Loading Buffer" (for colorless MasterMix), apply to the gel and run electrophoresis.

Recommended PCR assay

50µl PCR assay		Final Conc.
20µl	2,5XHFDBMasMix-3510	1X
0.2-1µM	each Primer	
Variable*	Blood sample	1-5%
To 50µl	PCR Grade Water	

*- depending on DNA template initial concentration

APPLICATIONS:

- High Fidelity (Hi-Fi) PCR
- "direct-blood" PCR
- GC-rich templates amplification
- "fast-PCR"

STORAGE CONDITIONS :

Store **2,5X HFDBMasMix** at -20°C (for long-term storage).

SHIPPING CONDITIONS:

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

General Protocol for amplification with 2,5X HFDBMasMix-3510

Add and mix the following components:

Component	50µL reactions	25µL reactions	Final concentration
PCR grade Water	Up to 50 µL	Up to 25 µL	
2,5X HFDBMasMix-1510	20 µL	10 µL	1X
Primers			0.3-0.5 µM each
Template DNA	optionally	optionally	5-50ng

2,5X HFDBMasMix contains 3,5mM (1X) of MgCl₂. For most applications, it working fine.

In some cases, we recommends to optimize Mg concentration in the range 3,5-5mM, depending on volume of blood and stabilizer type, what using in amplification.

We recommend using 50µl reaction for the PCR with **2,5XHFDBMasMix**

2,5X HFDBMasMix Components

1. HF-Fuzz Polymerase

2,5X HFDBMasMix contains optimal amount of enzyme for amplification in 50µl final reaction volume -1U. For most applications, including "long-distance" PCR amplification (DNA targets >5Kb for human genomic DNA) such quantity is optimal.

2,5X HFDBMasMix allows to amplify complex, GC-rich (up to 70% GC-pairs) DNA samples without addition of any, well-known PCR additives, with the highest speed (elongation time as low as 1,5 seconds per 100 b.p.).

2. Buffer Components

2,5X HFDBMasMix Buffer components especially designed to provide very high reproducibility across the wide range of amplification conditions with the blood samples as a target in amplification.

3. dNTP's

2,5X HFDBMasMix contains 200µM of each of regular dNTP's as final optimal concentration optimal. It is not necessary to optimize dNTP's concentration.

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 is better to use >= 20pmol of each primer.

5. PCR Additives

2,5X HFDBMasMix is compatible with the most of commonly used PCR additives for enhancing of high GC-content DNA templates (glycerol, betaine, DMSO and other), but it already contains all reagents for high quality PCR amplification of complex, GC-rich templates.

If one will use any additives, take into account the changes of T_m of primers and DNA to correct annealing temperature.

Cycling Protocol:

We recommend using for "direct blood" amplification from 1 to 5% of the blood as template per reaction volume, or 1mm² of the blood stain sample, for successful amplification.

Cycle step	2-step amplification		3-step amplification		Cycles
	T°C	Time	T°C	Time	
Initial Denaturation	98°C	1-5 min	98°C	1-5min	1
Denaturation	98°C	2-10 S	98°C	5-10 S	25-35
Annealing	-	-	55-72*	10-30 S	
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 T_m for the primer pair recommended as T_m of the lower primer, for the standard oligos <20nt.

For HF-Fuzz polymerase T_m of the primers should be corrected, as +3-10°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.

1. Denaturation:

-Initial denaturation for 5 min at 98°C is necessary only for blood cells lysis;

- we recommends using 98°C, as an optimal denaturation temperature, which allow to overcome problems with DNA templates with high level of secondary structures.

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

2. Annealing/Extension:

For **2,5X HFDBMasMix, which contains HF-Fuzz Polymerase "Annealing" and "Extension" steps**, should be combined if:

-T_m of both primers are not differs dramatically (<3°C);

-T_m of the primers are at least more than 62-64°C (optimal T_m for the primers lays between 65-70°C)

If primers T_m is about 60-62°C for both primers, ones can apply simple formula to determine starting Ta/e point - (T_m 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 15-20 sec per Kb for templates > 2 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.