DIALAT Ltd.

Data sheet
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2,5X HSMas^{GR}MIX-1510

RESEARCH USE ONLY

GREEN "Ready-to-Load" Hi-Fi amplification MasterMix

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

Stability:
2,5X HSMasMixGR stable for 24 months at -20ºC, **or** for 6 months at +4ºC storage without freezing.

CONTENT:
1X: HS-Fuzz Polymerase
0.2mM each of dNTP's
1,5 mM $MgCL_2$
Reaction buffer components
Stabilizer/enhancer
Two inherent dyes

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

DESCRIPTION
2,5X HSMasMixGR is a **green colored** ready-to-load premix of all components for "high- fidelity" amplification of target DNA, contains **stabilizer/ enhancer,** which improves thermostabilization of enzyme during PCR amplification and storage**.**
2,5X HSMasMixGR contains **HS-Fuzz** DNA Polymerase - a unique artificial enzyme created on the basis of intellectual protein design planning by genetic engineering technique. Activity of HF-Fuzz is blocked at ambient temperature (during PCR set-up) by highly specific **monoclonal antibodies to Pfu and Pfu-based** polymerases. 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 HSMasMixGR provides:
- Superior fidelity - 50x improvement compared to Taq polymerase;
- Excellent performance across a wide range of "difficult" templates;
- Long range amplification of complex targets - > 15 kb from genomic DNA;
- High speed - reduce reaction times (only 1-1,5 sec elongation time per 100bp)
- dUTP poisoning resistance
Just place it into the tube/plate adds primers and template of choice mix all components and run PCR.
After PCR reaction running, apply 5-10μl of reaction mixture directly without any other manipulations to the gel and run electrophoresis.

Recommended PCR assay

50μl PCR assay		Final Conc.
20μl	**2,5X HSMasMixGR-1510**	1X
0.2-1μM	each Primer	
Variable*	DNA Template	
To 50μl	PCR Grade Water	

*- depending on DNA template initial concentration

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

STORAGE CONDITIONS :
Store **2,5X HSMasMixGR** at -20°C (for long-term storage).

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General Protocol for amplification with 2,5X HSMasMixGR-1510

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 HSMasMixGR-1510	20 μL	10 μL	1X
Primers			0.3-0.5 μM each
Template DNA	optionally	optionally	5-50ng

2,5X HSMasMixGR contains 1,5mM (1X) of $MgCL_2$. For most applications, it working fine.
In some cases, we recommends to optimize Mg concentration in the range 2.0-3.0mM
We recommend using 25μl reaction for the PCR with **2,5X HSMasMixGRGR**

2,5X HSMasMixGR Components

1. HS-Fuzz Polymerase

2,5X HSMasMixGR 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 HSMasMixGR allows to amplify complex, GC-rich (up to 80% 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 HSMasMixGR Buffer components especially designed to provide very high reproducibility across the wide range of amplification conditions, including "fast-PCR" (reduced time of PCR reaction). In some cases we recommend to optimize Mg concentration in the range 1.5-4.5mM

3. dNTP's

2,5X HSMasMixGR 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 HSMasMixGR 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 Tm of primers and DNA to correct annealing temperature.

6. Dyes combination

2,5X HSMasMixGR contains two inherent dyes (orange and blue) which in combination gives green color. Both of them not reduce effective PCR amplification. They allows loading amplified sample directly to agarose gel for correct result identification.

Cycling Protocol:

We recommend to use for most applications 2-step PCR protocol, where primers of choice has Tm of both not less, than 61-62°C.

	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 +3-10°C, for the standard oligos <20nt.

1. Initial Denaturation:

-1) For most applications it is quite enough to denaturate sample in the first cycle for 1min for templates up to 3Kb and 2-5 min at 98°C for the targets more than 3 Kb.
Initial denaturation for 5 min at 98°C is necessary only for blood cells lysis;

2. Denaturation:

-1) 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 HSMasMixGR,** which contains **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 at least more than 62-64°C (optimal Tm for the primers lays between 65-70°C)

If primers Tm is about 60-62°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 15-20 sec per Kb for templates > 2 Kb.

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