DIALAT Ltd.

Data sheet
This data sheet provided for information only

2X UHotMas^{CF}Mix-1525

RESEARCH USE ONLY

Ready-to-use amplification MasterMix

Cat.No.	Pack	Conc.
MMUH-100	**100 rnx**	**2 X**
MMUH-500	**500 rnx**	**2 X**

Stability:
2X UHotMas^{CF}Mix stable for 24 months at -20°C, **or** for 6 months at +4°C storage without freezing.

CONTENT:
1X: UHotSmarTaq Polymerase
0.2mM each of dNTP's
1,5 mM $MgCL_2$
Reaction Buffer components
Stabilizer/enhancer

DESCRIPTION
2X UHotMas^{CF}Mix is a ready-to-use premix of all components for amplification of target DNA, contains **stabilizer/ enhancer,** which improves thermostabilization of enzyme during PCR amplification and storage**.**
2X UHotMas^{CF}Mix contains **blend of antibodies-blocked SmarTaq polymerase and special additive**, which are not active at ambient temperature (during PCR set-up) and activated automatically during the first PCR cycle at the temperature >70°C, preventing miss-priming and other artifacts formation.
It is no need for prolonged heating for activation of enzyme for PCR.
2X UHotMas^{CF}Mix contains optimized buffer reagents which greatly improve specificity of PCR with **complex**, **low-copy number DNA templates, multiplex PCR, "real-time" PCR ,**allowing to use very small initial quantities of DNA template.
One' can use an appropriate volume of
2X UHotMas^{CF}Mix for amplification reaction, depending on total final reaction volume.
Just place it into the tube/plate adds primers and template of choice mix all components and run PCR.
After PCR reaction running mix 5-10μl of reaction mixture with appropriative volume of "Loading Buffer" (for non-"real-time" mode PCR), apply to the gel and run electrophoresis.

Recommended PCR assay

50μl PCR assay		**Final Conc.**
25μl	**2X UHotMas^{CF}Mix 1525**	1X
0.2-1μM	each Primer	
Variable*	DNA Template	
To 50μl	PCR Grade Water	

*- depending on DNA template initial concentration

APPLICATIONS:
- Routine PCR
- Primer extension
- Real-Time PCR (all types)
- Low-copy PCR (UHotSmarTaq Polymerase)
- Multiplex PCR

STORAGE CONDITIONS :
Store **2XUHotMas^{CF}Mix** 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 2362, Fax. +499 613 4818 **e-mail**: info@dialat.ru

General Protocol for amplification with 2X UHotMas^{CF}Mix -1525

Add and mix the following components:

Component	50μL reactions	25μL reactions	Final concentration
PCR grade Water	Up to 50 μL	Up to 25 μL	
2XUHotMasCFMix-1525	25 μL	12.5 μL	1X
Primers			0.3-0.5 μM each
Template DNA	optionally	optionally	1-10ng

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 **2X UHotMas^{CF}Mix**

Cycling Protocol:

Cycle step	3-step amplification		Cycles
	T°C	Time	
Initial Denaturation	**95°C**	**1-2 min**	**1**
Denaturation	**95°C**	**10 S**	**25-35**
Annealing	**55-66***	**5-10 S**	
Extension	**72°C**	**15 Sec/Kb****	
Final extension	**72°C**	**1-2 min**	**1**
	4°C	**hold**	

*Optimal Tm for the primer pair recommended as Tm of the lower primer, for the standard oligos <20nt.
To optimize amplification we recommend using gradient PCR amplification, to rich final amplification conditions in the short time.
**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) strongly recommended to apply Extension time as 30 sec/Kb for the targets more than 1,5Kb

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