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## ALLin™ Mega HS HiFi Red Mastermix, 2X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0501	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HS HiFi Red Mastermix, 2X 3 x 1 ml - PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM MgCl <sub>2</sub> , enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading
HLM0505	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HS HiFi Red Mastermix, 2X 13 x 1 ml - PCR Water	

**Storage** In the dark at -20°C.

### APPLICATIONS

- Sequencing, including NGS library preparation
- Hot start PCR, multiplexing
- Direct gel loading option
- Fast high-fidelity PCR (up to 100 x *Taq*)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Blunt-end cloning and other applications

### PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HS HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to *Taq*. Compared to Mega HiFi, this hot start enzyme version allows for even higher sensitivity and specificity of PCR as well as for a room temperature reaction setup, and is excellent choice for multiplex reactions. The ALLin™ Mega HS HiFi DNA Polymerase is engineered to be much faster and to generate higher yield of long PCR products up to 20 kb from complex GC-rich templates. Therefore the ALLin™ Mega HS HiFi DNA Polymerase is an excellent choice for longer and very complex PCR applications where the highest fidelity is demanded. It is an enzyme of choice for cloning and all kind of sequencing applications including NGS. Generated blunt-ended PCR products are suitable for ligation into blunt vectors.

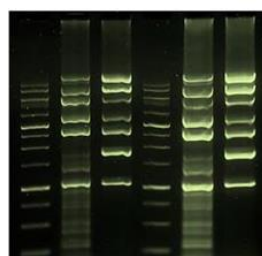
### BENEFITS

- Hot start enzyme for increased sensitivity and great multiplex results
- Fast, high yield PCR with the fidelity 100x higher than *Taq*
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS library prep. and other sequencing applications
- Red master mix for direct gel loading, supplied with water

### PERFORMANCE

The convenience of ALLin™ Mega HS HiFi DNA Polymerase is maximized by the use of 2X Red Mastermix providing the advantage of reduced pipetting and direct gel loading.

ALLin™ Mega HS HiFi Red Mastermix, 2X is premixed with red dye and density reagents for direct loading on the gels after the PCR. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.



High sensitivity multiplex PCR results achieved with **Allin™ Mega HS HiFi DNA Polymerase**

Gel analysis of multiplex PCR reactions - compared to competitor enzyme (2; 5), the Allin™ Mega HS HiFi DNA Polymerase (3; 6) gives more specific multiplex result.

L 2 3 L 5 6

### PROTOCOL

- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix very well before use. Mixing of the mix is very important for the final yield!
- For complex, GC rich templates, use 99-100°C denaturation temperature, it might help to increase the yield.
- For established PCRs, try two-step cycling protocol with a combined annealing-denaturation step of 70°C (68°C to 75°C).
- Run an annealing temperature gradient (2°C increments) from 60°C to 66°C to choose the best conditions.
- The longer the amplicon, the longer the extension time: depending on the complexity of the template, perform extension from 10 sec/kb to 30 sec/kb. Longer extension for more complex templates is needed. For multiplexing, start with extension time needed for the longest fragment.

- ✓ Prepare a 50 µl reaction:

Rev. & For. Primers	To 0.2 - 0.6 µM each (~2µl of 10 µM)
cDNA Template <i>or</i>	<100 ng <i>or</i>
gDNA Template	10 - 200 ng
ALLin™ Mega HS HiFi Red Mastermix, 2X	25 µl
PCR Water	to 50 µl

- ✓ Mix gently, avoid bubbles.

- ✓ Place into the instrument set like:

Initial denaturation	1 cycle: 95°C - 1 min
Denaturation	25-35 cycles: 95°C - 15 sec
Annealing	25-35 cycles: 60-66°C - 15 sec
Extension	25-35 cycles: 72°C - 30 sec (10-30 sec/kb)

- ✓ Store probes for short time on ice, for long at -20°C.
- ✓ Load probes directly on the agarose gel. ALLin™ Mega HiFi Red Mastermix, 2X is premixed with red dye and density reagents for direct loading after the PCR. In a 2% agarose TAE gel the dye migrates with ~350 bp DNA, in 1% agarose - with ~ 600 bp DNA.

IN VITRO RESEARCH USE ONLY

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