Check the product label for actual catalog number, lot and expiry date.

ALLin™ Mega HiFi Mastermix, 2X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
HLM0201	100 r of 50 µl	2 x 1.25 ml - ALLin™ Mega HiFi Mastermix, 2X 3 x 1 ml – PCR Water	1X mastermix contains 1 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers
HLM0205	500 r of 50 µl	10 x 1.25 ml - ALLin™ Mega HiFi Mastermix, 2X 13 x 1 ml – PCR Water	
Storage	*In the dark at -20°C.*		

APPLICATIONS

- Fast high-fidelity PCR (up to 100 x *Taq*)
- Long PCR up to 20 kb
- Amplification of complex (GC/AT rich) templates
- Sequencing, including NGS
- Blunt-end cloning and other applications

BENEFITS

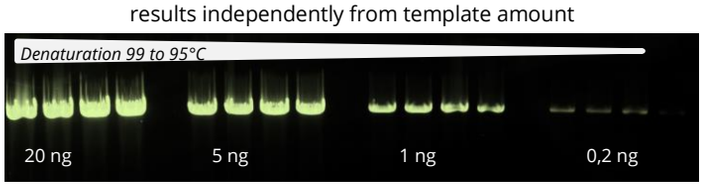
- Fast, high yield PCR with the fidelity 100x higher than *Taq*
- Up to 20 kb long PCR even from complex templates
- Increased processivity for faster amplification and higher yield
- High thermostability for better denaturation of GC rich templates
- Best choice for NGS and other sequencing applications
- Master mix format for maximum convenience, supplied with water

PRODUCT DETAILS

Derived from our HiFi polymerase, the highQu ALLin™ Mega HiFi DNA Polymerase provides much lower error rate PCR with a 100 higher fidelity compared to *Taq*. The ALLin™ Mega 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 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.
The convenience of ALLin™ Mega HiFi DNA Polymerase is maximized by the use of 2X Mastermix providing the additional advantage of reduced pipetting and minimized errors.

PERFORMANCE

Applying fast cycling conditions and high denaturation temperatures, ALLin™ Mega HiFi DNA Polymerase provides consistent long PCR results independently from template amount



Amplification of ~6 kb fragment from mouse gDNA using ALLin™ Mega HiFi DNA Polymerase at different template concentrations from 20 ng down to 0,2 ng, at different denaturation temperatures for each concentration from 99°C down to 98, 97, 95 °C. Fast cycling protocol -10 seconds per kilobase: Denaturation 1 min at 95°C, 30 cycles: 10 sec 95-99°C; 10 sec 67°C; 60 sec 72°C, 2 min 72°C.

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.

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	To 0.2 - 0.6 µM each (~2µl of 10 µM)	
cDNA Template *or* gDNA Template	<100 ng 10 - 200 ng	*or*
ALLin™ Mega HiFi 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.

IN VITRO RESEARCH USE ONLY

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