www.behgene.com
Tel. (+98) 905 363 3840
E-mail: info@behgene.com

Complex PCR Master Mix(2X)

1. PRODUCT DESCRIPTION

The **Complex PCR Master Mix(2X)** from Behgene is a 2X master mix optimised to achieve maximum yield and specificity in amplification reactions using complex (genomic DNA, GC-rich templates, templates with secondary structures) and/or scarce DNA templates.

Genomic DNA, GC-rich templates or those with a secondary structure are difficult to amplify. The main reason for this difficulty is that conventional polymerases introduce errors during DNA replication that can stop the amplification reaction, thereby resulting in truncated products and/or error accumulation in the amplified sequence and compromising the yield and fidelity of the PCR.

The Complex PCR Master Mix overcomes these difficulties by incorporating a mix of highly efficient enzymes, including HotSplit DNA Polymerase, which has a hot start effect, and *Pfu* DNA Polymerase, which has proofreading activity. The combination of these enzymes, together with the special reaction buffer and cofactors included in the mix, results in high yield and copy fidelity in complex amplification reactions. The hot start nature of the HotSplit DNA Polymerase contributes significantly to the specificity achieved with the Complex PCR Master Mix by reducing the formation of primer dimers and nonspecific amplification products.

Applications of Complex PCR Master Mix:

- ✓ *Amplification of GC-rich DNA templates*
- ✓ *Amplification of DNA templates rich in secondary structures*
- ✓ *Amplifications using tiny amount of template (detection limit of 0.1 pg)*
- ✓ *Amplification of fragments up to 10 kb (genomic DNA)*
- ✓ *Amplification of fragments up to 30 kb (lambda DNA)*

Components: HotSplit DNA Polymerase; *Pfu* DNA Polymerase; dNTPs: $MgCl_2$; reaction buffer; adjuvants and stabilisers at concentrations suitable for performing a wide range of DNA amplification reactions.

2. STORAGE CONDITIONS

Store vials of Complex PCR Master Mix at **-20ºC** in a freezer that ensures a constant temperature (frost-free freezers are not recommended). If the kit is to be used frequently, we recommend to prepare aliquots to avoid frequent freeze/thaw cycles.

If handled and stored according to these recommendations, the stability of this product will be as indicated on the corresponding label.

REF.	FORMAT	CONTENTS
3040	50 rxn of 25 µL	DNA AmpliTools Complex Master Mix
3050	100 rxn of 25 µL	DNA AmpliTools Complex Master Mix

Store at -20ºC

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3. GUIDELINES FOR USING THIS PRODUCT

Template: The quality of the DNA template is key to obtaining optimal results in amplification reactions. Although conventional extraction methods produce templates with sufficient quality for PCR, some of the reagents used during purification (phenol, EDTA, proteinase K, ionic detergents, silica particles, etc.) often inhibit amplification. Behgene recommends its Speedtools product range for the extraction and purification of genomic DNA from blood (*Behmag DNA Extraction*); tissue (*Behmag Tissue DNA Extraction kit*); gel (*Behmag gel DNA Extraction kit*).

Samples should be transported cold as a lack of refrigeration may lead to degradation of the DNA. All clinical samples must be handled as though they were potentially infectious.

The quantity of DNA to be included in the amplification reaction will depend on the source and quality of the template to be used. We recommend 0.2-10 ng for low-complexity DNA (e.g. plasmid, lambda or bacterial DNA) and 10-250 ng for complex templates (human genomic DNA); use 1 ng and 50 ng, respectively, for initial optimisation. An excess of template increases the formation of nonspecific amplification products and decreases the yield of the reaction.

$MgCl_2$ concentration: The concentration of magnesium ions affects primer annealing and denaturation of the template, as well as polymerase activity and fidelity. High $MgCl_2$ concentrations can result in the formation of nonspecific amplification products and low concentrations can lower the yield of the reaction. The final $MgCl_2$ concentration Complex PCR Master Mix 2X is **2 mM,** an ideal concentration for the majority of amplicons.

Primer design: The primers used in the amplification reaction are usually around 15-30 bases long and have a GC content of 40-60%. Moreover, the annealing temperatures for both primers should be practically identical.

When performing the design, remember that the primers should not form forks or be mutually complementary. The absence of complete homology with the DNA template at the 5' end of primers is not as critical as the lack of complementarity at the 3' end. Avoid including more than three G or C nucleotides at the 3' end of the primers in order to reduce nonspecific annealing.

The optimal quantity of DNA and primers in the PCR must be determined experimentally for each new template/primer combination. The optimal concentration for complex amplifications using Behgene Complex PCR Master Mix is in the range **0.3-0.7 µM**; use 0.5 µM of each primer for initial optimisation.

PCR program: Some program parameters, including the denaturation, annealing and elongation temperature and time and the number of cycles, affect both the amplification specificity and efficiency. Variations in the size of the amplification product, the source of the template and primer sequence usually require changes in the amplification program.

A two-step amplification protocol can be applied when primers with an annealing temperature higher than 60 ºC are used.

مستر میکس کمپلکس به ژن

ویستا زیست فناوری به ژن

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