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DNA AmpliTools Multiplex Master Mix

REF.	FORMAT	CONTENT
10441	100 rxns of 20 µl	DNA AmpliTools Multiplex Master Mix
10442	200 rxns of 20 µl	DNA AmpliTools Multiplex Master Mix

Store at -20ºC

Research Use Only. Not for use in diagnosis procedures

Notice to users: Some of the applications which may be performed with this product are covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application

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1. PRODUCT DESCRIPTION

The new developed **DNA AmpliTools Multiplex Master Mix (2X)** is a sensitive, premix solution for performing simultaneous amplification of multiple targets using multiple primers. It has been designed to reduce PCR reaction time and to enable an efficient use of valuable samples.

The DNA AmpliTools Multiplex Master Mix has been developed by Biotools including Biotools HotSplit DNA Polymerase and the new Reaction Buffer in a convenient master mix format that minimises nonspecific amplification products and primer-dimers. This mix is recommended for amplifications up to ***7 kb in length***.

The ready to use format dramatically reduces set up time and liquid handling steps, thereby minimising the risk of contamination. With the exception of primers and template, the convenient DNA AmpliTools Multiplex Master Mix (2X) provides all the necessary reagents at optimal concentrations to perform multiplex PCRs without previous optimization.

The DNA AmpliTools Master Mixes of Biotools allow convenient and easy to use PCRs, and they are ideally suited for routine PCR applications.

Applications of *DNA* AmpliTools Multiplex Master Mix:

- √ *Amplification of multiple fragments for SNPs genotyping*
- √ *High specificity PCR (up to 7 kb)*
- √ *Typing of transgenic organism.*
- √ *Generation of PCR products for cloning*
- √ *Detection of pathogen*

Note: *This master mix is not recommended for certain experiments dealing with amplification of sequences homologous to those found in E. coli.*

Components: The DNA AmpliTools Multiplex Master Mix contains Biotools HotSplit DNA Polymerase, dNTPs, 3.0 mM $MgCl_2$, and Reaction Buffer optimised to carry out multiplex amplification reactions.

2. STORAGE AND HANDLING INSTRUCTIONS

Store DNA AmpliTools Multiplex Master Mix tubes at **-20 ºC** in a constant temperature freezer (frost-free freezers are not recommended). The mix must be thawed and handled on ice. For frequent use, divide in aliquots to avoid multiple freeze-thaw cycles.

If stored under the recommended conditions, the product will maintain performance through the indicated date on the label.

3. GENERAL CONSIDERATIONS

Template: DNA condition is a key point to obtain optimal results. DNA prepared using standard isolation techniques is a suitable substrate for amplification. Nevertheless, even trace amounts of certain agents used in DNA purification procedures (phenol, EDTA, proteinase K, ionic detergents, silica particles, etc.) often inhibit amplification. We recommend the use of our Speedtools line for extraction of genomic DNA from blood (*Speedtools DNA Extraction*); from tissue (*Speedtools Tissue DNA Extraction kit*); from food (*Speedtools Food DNA Extraction kit*); and from plant material (*Speedtools Plant DNA Extraction kit*).

Samples should be transported and stored frozen. In samples that have been stored without refrigeration, DNA can be degraded. In case of working with clinical samples, handle them as if they are capable of transmitting infectious agents.

The quantity of template DNA to be added in each reaction depends on the source and quality of the template. For the DNA AmpliTools Multiplex Master Mix we recommend **25ng - 500ng** for complex templates such as genomic DNA. Higher amounts of template increase the formation of non-specific products and may reduce PCR yield. If you do not know the concentration of DNA, add a fixed volume of the extraction mixture to the problem samples.

Primer design: Primers typically are 15-30 bases in length and contain approximately 40-60% GC-content: the annealing temperatures of primer pairs should be nearly identical.

Care must be taken to design primers that do not form hairpin loop structures or are self-complementary. The 5´ end of a primer may contain mismatches between the primer and template, whereas this is not recommended at the 3´ end. Avoid placing more than three G or C nucleotides at the 3' end to lower the risk of non-specific priming.

The optimal quantity of template and primers must be determined empirically for each combination of template and primer. The recommended concentration range of each primer is 0.2-1.0 µM. A concentration of **0.5 µM** (each primer) works for most Multiplex PCR amplifications.

Additives: Amplification of some difficult targets, like GC- rich sequences, may improve with additives, such as DMSO or betaine. The DNA AmpliTools Multiplex Master Mix is compatible with common PCR additives.

Cycling Parameters: Many parameters influence both the specificity and efficiency of amplification including the temperature and duration of denaturation, annealing and elongation; and total cycle number. Variations to the program may be required depending on the amplicon size, the template, etc

The DNA AmpliTools Multiplex Master Mix allows PCR cycling times can be reduced **from 40% to 80%**, depending on the length of the expected amplicon, as well as the complexity of your starting template.

-Denaturation:

- Initial denaturation: No separate activation step is required to activate the HotSplit DNA polymerase. An initial denaturation of 5 min at 94-96 °C is enough for template DNA with ≤ 50% in GC-content and 5-10 min for genomic DNA or DNA with > 50% in GC-content.
- Subsequent denaturation steps will be between: 10-30 seconds at 95-98 °C.

-Annealing:

- Annealing temperature should be 5 °C lower than the melting temperature of primers.
- The annealing step is between 10-30 seconds..

-Extension:

- The optimal extension temperature for Biotools HotSplit DNA Polymerase is 72°C. For larger products, the extension temperature should be reduced at 68 °C.
- The recommended extension step is **10-25 seg/kb** of the expected amplicon.
- A final extension of 10 seconds at 72°C is recommended to fill-in any possible incomplete reaction products.

Cycle Number:

- Generally, 25-30 cycles result in optimal amplification of desired products. Occasionally, up to 35 cycles may be performed, especially for detection of low-copy targets.

Reaction Conditions: The DNA AmpliTools Multiplex Master Mix (2X) should be used at 1X concentration with template and primers in a final reaction volume of 15, 20, or 25 µl. The recommended reaction volume is **20 µl**.

4. STANDARD PROTOCOL

Materials to be supplied by user:

- Downstream oligonucleotide primer
- Upstream oligonucleotide primer
- Nuclease-free water
- Template DNA

Laboratory workflow must be unidirectional, from pre-amplification to amplification areas. Specific equipment for each working area must be used, in order to avoid cross contaminations.

1. Thaw the DNA AmpliTools Multiplex Master Mix, primers and DNA template.

2. Calculate the number of needed reactions; do not forget to include a no template control (NTC) reaction to check for contamination.

3. Mix the DNA AmpliTools Multiplex Master Mix (2X) before use in order to prevent localised concentrations of salts; then spin it briefly. .

Note: *Due to the hot start nature of the enzyme, reactions can be prepared at room temperature. The HotSplit DNA Polymerase is activated during the initial denaturation step.*

4. Add the following components for each reaction

Recommended amount of template and primers

Component	***Final concentration***
DNA AmpliTools Multiplex Master Mix (2X)	1X
Forward primer	0.5 µM (0.2-1.0 µM)
Reverse primer	0.5 µM (0.2-1.0 µM)
Template DNA*	Plasmid: 1 ng-10 ng gDNA: 1-500 ng

***** *Start with 1 ng or less o menos for simple templates such as plasmid or lambda , or 10 ng for complex templates such as gDNA*

Note: *The optimal quantity of template and primers must be determined empirically for each new combination of template and primer. The reaction conditions described in this protocol are general recommendations only.*

5. Mix the reaction by vortexing and spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.

6. For thermal cycler without heated lid overlay add a mineral oil layer.

7. Transfer PCR tubes to a thermal cycler with the block preheated to 95 °C and perform the selected PCR program.

Standard Amplification Program

Cycle Step	Nº Cycles	Temperature	Time
Initial Denaturation	1	95 ºC	5 min
Denaturation	30-45	95-98 ºC	3-30 sec
Annealing		T_m-5 °C	3-30 sec
Extension		68-72 ºC	20 sec/kb#
Final Extension (optional)	1	68-72 ºC	3-10 min
Cooling	∞	4 ºC	∞

Use an extension time of 20 sec per kb DNA (*ej. 5 s for 250 bp, 10 s for 500 bp, 30 s for 1.5 kb, 90 s for fragments larger than 1.5 kb).*

8. Load 25-50% of the reaction mixture on agarose gel to analyse the PCR product.

5. TROUBLESHOOTING

Little or no amplification detected

1. **Check the quality of your template DNA by agarose gel electrophoresis or fluorimetry**. Some DNA purification procedures, particularly genomic DNA isolation, can result in the purification of inhibitors. Reduce the volume of template DNA in reaction or dilute the template prior to adding to reaction. Ethanol precipitation of DNA and repetitive treatments of DNA pellets with 70% ethanol is usually effective in removing traces of contaminants from the sample.
   Sample DNA damaged or degraded. Use sample that has been processed and stored properly to minimise shearing and nicking. Use of excess template can also reduce PCR yield
2. **Redesign primers.** Design primers that have higher annealing temperatures and do not form hairpin loops or primer-dimers.
3. **Optimize primer concentration.** Although lower primer concentration can prevent primer-dimer formation, sufficient primers are needed for successful PCR. The concentration of each primer should be 0.2 µM. Increase this concentration in increments of 0.1 µM. Primer concentration should not surpass 1.0 µM.
4. **HotSplit DNA Polymerase not activated.** Check whether PCR was started with an initial incubation step at 95 °C for 5 min
5. **Increase initial template denaturation time.** Increase the length of initial template denaturation. It is very important in the early cycles to make sure that the template is completely denatured. Templates rich in GC-content or with secondary structures often require longer initial denaturation (≤ 10 min).
6. **Lower annealing temperature.** Lower the annealing temperature in 2 °C decrements. Remember that PCR additives and enhancing agents usually affect the annealing temperature of primers
7. **IIncrease number of cycles.** Perform additional cycles in increments of 5 cycles. Occasionally, up to 35-40 cycles may be performed, especially for detection of low-copy targets.
8. **Change extension phase conditions.** Increase the extension time by increments of 5 sec. For large products reduce the extension temperature up to 68 °C.
9. **Add additives to the reaction mixture.** Adding PCR enhancing agents (e.g. DMSO or betaine) or general estabilising agents (e.g. albumin) may improve yield.

Multiple products or a smear detected

1. **Decrease concentration of reaction components.** Check the concentration of template DNA by agarose gel electrophoresis or fluorimetry. Decrease the amount of DNA and/or primer added to the reaction.
2. **Sample DNA damaged or degraded.** Use sample that has been processed and stored properly to minimise shearing and nicking. Low template quality templates or higher amounts of template increase the risk of generation of non-specific PCR products
3. **Check the primers for degradation.** Check by electrophoresis in a denaturing acrylamide gel.
4. **Redesign primers.** Design primers that have higher annealing temperatures and do not form hairpin loops or primer-dimers.
5. **Increase annealing temperature.** Increase the annealing temperature in 2°C increments.
6. **Decrease number of cycles.** Decrease number of cycles in decrements of 5 cycles.
7. **Carryover contamination.** If the no template control (NTC) shows PCR product or a smear, exchange all reagents.

6. ORDERING INFORMATION

DESCRIPTION	Format	Size	Reference
DNA AmpliTools Multiplex Master Mix	100 rxns of 20 µl	1 x 1100 µl	10441
DNA AmpliTools Multiplex Master Mix	200 rxns of 20 µl	2 x 1100 µl	10442