Produced by:
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BIOTOOLS B&M LABS.S.A.

DNA AmpliGel Master Mix-Plates

REF.	FORMAT	CONTENT
10.545	10 x 96-well plates	DNA AmpliGel Master Mix-Plates
10.546	20 x 96-well plates	DNA AmpliGel Master Mix-Plates

Store at 4ºC

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.

Ed .03 –February 2016

1. PRODUCT DESCRIPTION

The **DNA AmpliGel Master Mix-Plates** is a pre-mixed and pre-dispensed master mix for performing PCR amplifications of different templates. DNA AmpliGel Master Mix is suitable for targets up to *5 kb in length*.

With the exception of primers and template, the DNA AmpliGel Master Mix-Plates provide all the necessary reagents at optimal concentrations to perform **50 µl** PCRs.

DNA AmpliGel Master Mix-Plates is available pre-dispensed into 96-well plates. The gel format minimises handling steps and reduces risk of contaminations; only primers and DNA have to be added. The use of this master mix offering virtually no reaction set up time.

DNA AmpliGel Master Mix is designed and manufactured using a Biotools's technology, "Gelification Technology", which is covered by the international patent PCT/ES02/00109. Biotools gelified master mixes represent a step forward in respect to their liquid counterparts because they are stored at 4 °C, and can be shipped and handle at room temperature*.

Advantages of gelification technology*:

- √ *Minimal set-up time*
- √ *Less errors*
- √ *Cost-effective (minimal budget for consumables)*
- √ *Convenient store and shipment conditions*
- √ *Minimal intra- and inter-assay variation*

The DNA AmpliGel Master Mixes of Biotools allow for convenient and easy to use PCR and they are ideal for a wide variety of amplifications.

Applications of DNA AmpliGel Master Mix:

- √ *Routine PCR with high reproducibility (up to 5 kb)*
- √ *Primer extension*
- √ *TA cloning*
- √ *Gene sequencing*
- √ *RT-PCR*
- √ *High throughput PCR*
- √ *Field experiments*

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

Components: DNA AmpliGel Master Mix-Plates contains recombinant Biotools DNA Polymerase; dNTPs; $MgCl_2$; Reaction Buffer; and stabilisers at optimal concentrations for efficient DNA amplifications.

2. STORAGE AND HANDLING INSTRUCTIONS

Store DNA AmpliGel Master Mix plates at **4 ºC,** in a humidity controlled atmosphere. If stored under the recommended conditions, the product will maintain performance through the indicated date on the label.

Shipping and handling can be performed at room temperature.

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. We recommend **1 pg-10 ng** for a simple template such as plasmid and phage DNA, and between **1 ng-500 ng** for complex templates such as genomic DNA. Higher amounts of template increase the formation of non-specific products and may reduce PCR yield; avoid templates amounts >1 µg. If you do not know the concentration of DNA, add a fixed volume of the extraction mixture to the problem samples.

$MgCl_2$ Concentration: Magnesium ion concentration affects primer annealing and template denaturation, as well as DNA polymerase activity and fidelity. Generally, excess Mg^{2+} results in accumulation of non-specific products, whereas insufficient Mg^{2+} results in reduced yield of the desired PCR product. When gel is reconstituted to a **50 µl** final volume, the concentration of $MgCl_2$ is **2 mM** (optimal for most amplification reactions).

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. Excessive primer increase the probability of mispriming and generation of primer-dimer or non-specific products. In the range of **0.2 µM to 0.5 µM** work for most amplification; use 0.2 µM of each primer as starting point of optimisation.

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.

-Denaturation:

- Initial denaturation: 5 min at 94-96 °C for template DNA with ≤ 50% in GC- content and 6-10 min for genomic DNA or DNA with > 50% in GC-content.
- Subsequent denaturation steps will be between 5-60 seconds at 94-96 °C.

-Annealing:

- Annealing temperature should be 5 °C lower than the melting temperature of primers.
- The annealing step is between 15-60 seconds.

-Extension:

- The optimal extension temperature for Biotools DNA Polymerase is 72°C. For larger products, the extension temperature should be reduced at 68 °C.
- The recommended extension step is 1 min/kb of the expected amplicon.
- A final extension of 3-10 min at 72°C is recommended to fill-in any possible incomplete reaction products.

-Cycle Number:

- Generally, 25-35 cycles result in optimal amplification of desired products. Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

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.

Proceed to the Reagent Preparation Area in a laminar flow cabinet. Wear disposable gloves and use sterile and nuclease free plastic material in order to avoid contaminations and false negative results. We also recommend the use of filter tips.

1. Thaw primers and template DNA on ice.

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

3. Remove the desired quantity of gelified plates. Examine these plates to verify that DNA AmpliGel Master Mix is not rehydrated.

4. Remove the plate sealer film.

5. Add primers and template DNA into the DNA AmpliGel Master Mix wells.

6. Complete with distilled nuclease free water to a final volume of **50 µl**. *Do not calculate any volume for the gel.*

Recommended amount of template and primers

Component	***Final concentration***
Forward primer	0.2-1.0 µM
Reverse primer	0.2-1.0 µM
Template DNA*	Plasmid: 1 pg-10 ng gDNA: 1-500 ng

**Start with 20 pg, for a simple template such as plasmid and phage DNA or 20 ng, for complex templates such as gDNA.*

Recommended amount of template and primers

Component	***50 µl rxn***
Forward primer	10-50 pmol (x µl)
Reverse primer	10-50 pmol (x µl)
Template DNA*	variable (x µl)
Sterile bidistilled water	to a final volume of **50 µl**

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.*

7. Mix the well contents by shaking plates carefully.

Note: *To achieve a **Hot Start effect**, not resuspend the gel. Gelified reagents will be dissolved during initial denaturation step.*

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

9. Close plates by using new sealer films.

Standard Amplification Program

Cycle Step	Nº Cycles	Temperature	Time
Initial Denaturation	1	94 ºC	5 min
Denaturation	25-35	94 ºC	15-60 sec
Annealing		T_m-5 °C	15-60 sec
Extension*		72-74 ºC	60 sec/kb
Final Extension (optional)	1	72-74 ºC	3-10 min
Cooling	∞	4 ºC	∞

10. Load 10-30 µl of the reaction mixture on agarose gel to analyse the PCR product.

5. TROUBLESHOOTING

Little or no amplification detected

1. **Check template quality and quantity.** 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. **Optimise primer concentration.** Although lower primer concentration can prevent primer-dimer formation, sufficient primers are needed for successful PCR. Increase primer concentration in increments of 0.1 µM.
4. **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).
5. **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.
6. **Increase number of cycles.** Perform additional cycles in increments of 5 cycles. Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.
7. **Change extension phase conditions.** Increase the extension time by increments of 30 sec. For large products reduce the extension temperature up to 68 °C.
8. **Add additives to the reaction mixture.** Adding PCR enhancing agents (e.g. DMSO or betaine) may improve yield. General estabilising agents (e.g. albumin) also may help to overcome amplification failure.

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. **DNA AmpliGel Master Mix rehydrated.** Discard any plates that appear in bad conditions.

6. ORDERING INFORMATION

DESCRIPTION	Format	Reference
DNA AmpliGel Master Mix-Plates	10 x 96-well plates	10.545
DNA AmpliGel Master Mix-Plates	20 x 96-well plates	10.546
DNA AmpliGel Master Mix-Strips	12 x 8-tube strips	10.541
DNA AmpliGel PLUS Master Mix-Strips	12 x 8-tube strips	10.551