ProductInformation

OptiStrips™ Mg
For PCR Optimization

Product No. **M1932**
Store at room temperature

Product Description

There are many parameters that can influence the specificity, fidelity and yield of a PCR product. Magnesium ion concentration has been shown to influence the primer template annealing temperature[2], fidelity[3], specificity and yield of PCR[2,4]. Sigma's OptiStrips™ Mg are designed to simplify the magnesium ion optimization in a PCR reaction. Standard PCR† protocols call for a final magnesium ion concentration of 1.5 mM[1]. However, the presence of EDTA and other chelating agents often found in template DNA can affect the concentration of free magnesium in the PCR. For this and other reasons it is necessary to optimize specific PCR applications with respect to this divalent cation[1,4,5,6]. The optimum magnesium concentration for *Taq* DNA polymerase is generally found to lie between 2-4 mM. This range also overlaps the working magnesium concentrations of Tth and pfu polymerases.[7] This range assumes an overall dNTP concentration of 0.8 mM. Sigma's OptiStrips Mg cover concentrations from 1-4 mM to accommodate reactions with slightly lower dNTP concentrations.

Reagents

- OptiStrips Mg, 8-tube strip, 6 or 12 strips per package

Reagents and Equipment Required But Not Provided
(Sigma product numbers have been given where appropriate)

- Water, Product No. W1754
- Mineral Oil, Product No. M8662
- 10 mM dNTP mix, Product No. D7295
- Primers
- DNA to be amplified (template)
- Thermal cycler
- *Taq* DNA polymerase without Magnesium, Product No. D4545 **or**
- *RedTaq* DNA polymerase without Magnesium, Product No. D2812

Precautions and Disclaimer

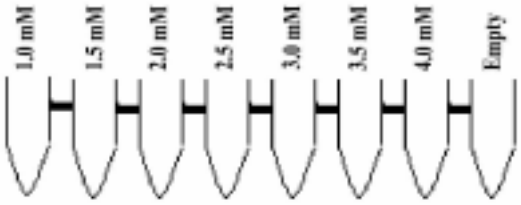
Sigma's OptiStrips Mg are for laboratory use only. Not for drug, household or other uses.

Storage

OptiStrips Mg can be stored at room temperature. Freezing for long term storage (more than 2 months) is recommended. Performance will not be adversely affected by multiple freeze-thaw cycles.

Procedure

Included in each OptiStrips Mg are seven solutions which, when brought to a final volume of 50 µl, will give concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mM $MgCl_2$, respectively. The last well of the 8-tube strip is left empty for easy alignment of the tubes (see below).



The total volume in each of the filled tubes is 15 µl. Assembly of a typical reaction is as follows:

Component	Volume	Final Concentration
$MgCl_2$	15 µl in strip	1-4 mM
10X PCR buffer II, Product No. P2317	5 µl	1X
10 mM dNTP mix	1 µl	0.2 mM each
Template and primers	---	See note*
Water	X µl	
5 un/µl Taq DNA polymerase	0.5 µl	2.5 units
50 µl Total volume		

* Note: Primers are typically added to 0.1-0.5 µM final concentration. Template can range from 50 ng for genomic DNA to less than 100 template copies.

An optimized PCR using an appropriate cycling protocol and well designed primers should produce a single DNA band in high yield as judged by agarose gel electrophoresis.[8]

The table below (Table 1) is provided to enable the user to replicate for future PCR amplifications the magnesium concentration determined to be optimal.

Taq™ is a registered trademarks of Perkin Elmer Corp., Norwalk, CT.

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

References

1. Saiki, R.K.; "The Design and Optimization of the PCR" in PCR Technology, Principles and Applications for DNA Amplification, Erlich H. A. ed; Oxford University Press, pp. 7-8 (1992)
2. Kidd, K.K. and Ruano, G.; "Optimizing PCR" in PCR 2: A Practical Approach, McPherson, M.J., Hames, B.D., and Taylor, G.R., eds; The Practical Series Approach Series, Rickwood, D. and Hames, B.D., series eds; IRL Press © 1995, pp. 1-22
3. Fromant, M., *et al.*; "Effect of dNTP and Divalent Metal Ion Concentrations on Random PCR Mutagenesis" in Genetic Engineering with PCR, Horton, R.M and Tait, R.C. eds.; Horizon Scientific Press, pp. 39-55 (1998)
4. Oste, C., "Optimization of magnesium concentrations in the PCR reaction", Amplifications Forum PCR Users, **1**, 10 (1989)
5. Innis, M.A. and Gelfand, D.H.; "Optimization of PCRs" in PCR Protocols, A Guide to Methods and Applications, Innis, M.A, Gelfand, D.H., Sninsky, J.J. and White, T.J. eds.; Academic Press © 1990, pp. 3-12
6. Powell, S.J.; "Protocol Optimization and Reaction Specificity" in PCR, Essential Data; Newton, C.R. ed.; Essential Data Series, Rickwood, D. and Hames, B.D. series eds. John Wiley & Sons; pp. 72-86 (1995)
7. Bej, A. K. and Mahbubani, M. H.; "Thermostable DNA Polymerases for in Vitro DNA Amplifications" in PCR Technology, Current Innovations, Griffin, H.G. and Griffin, A.M. eds.; CRC Press pp. 221-237 (1994)
8. Sambrook, J., *et al.,* Molecular Cloning, a Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press © 1989, pp. 6.3-6.19

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Table 1

For optimal magnesium concentration, add the appropriate amount of 25 mM magnesium chloride solution (Product No. M8787) based on the results of the OptiStrips Mg, indicated in the table below. The table provides a variety of reaction volumes. Add the appropriate amount of magnesium based on your reaction volume.

Reaction Volume	Desired Final Magnesium Chloride Concentration						
	1.0 mM	1.5 mM	2.0 mM	2.5 mM	3.0 mM	3.5 mM	4.0 mM
100 µl	4.0 µl	6.0 µl	8.0 µl	10.0 µl	12.0 µl	14.0 µl	16.0 µl
50 µl	2.0 µl	3.0 µl	4.0 µl	5.0 µl	6.0 µl	7.0 µl	8.0 µl
25 µl	1.0 µl	1.5 µl	2.0 µl	2.5 µl	3.0 µl	3.5 µl	4.0 µl
20 µl	0.8 µl	1.2 µl	1.6 µl	2.0 µl	2.4 µl	2.8 µl	3.2 µl

NOTE: Taq and REDTaq have different apparent magnesium optima. Optimized reaction conditions for Taq DNA polymerase will not be the same for REDTaq DNA polymerase.