Magnesium Chloride (25 mM MgCl2)

Technical Data Sheet

Catalogue	Pack Size
MC-1	2.5 mL

For research and educational use only.

Description

Magnesium ions, added in the form of magnesium chloride ($MgCl_2$), are an essential cofactor for polymerase enzyme activity during PCR. Too low a concentration will result in weak amplification or complete PCR failure, while too much can promote non-specific amplification.

PCR Master Mixes contain enough $MgCl_2$ for typical use but they may require an increased concentration in some circumstances. For example when compensating for PCR inhibitors present in DNA extracts that might bind to Mg^{2+} ions.

$MgCl_2$ solution is used for adjusting Mg^{2+} concentration in PCR reactions. It can be used in combination with Master Mixes such as FIREPol® and HOT FIREPol® Blend Master Mixes, or other commercial Master Mixes, PCR beads and polymerases. In most applications a final concentration of 1.5 mM $MgCl_2$ is optimal for a satisfactory yield, but some PCR reactions may require up to 4.5 mM $MgCl_2$ or more.

Application Recommendations

- PCR reactions
- Enzymatic reactions

Reagent Composition

Magnesium chloride solution (25 mM $MgCl_2$).

Storage & Stability

Routine storage at –20ºC. Temporary storage at room temperature has no detrimental effects on the quality of this reagent. Optionally aliquot into sterile tubes and freeze to minimise potential microbial contamination after opening.

Shipping conditions

Shipped at room temperature**.** Shipping at room temperature has no detrimental effects on the quality of this reagent.

Safety warnings and precautions

This product and its components are not considered hazardous in their given concentrations. However, as with all scientific reagents this product should be handled and stored with care as standard practice. Wear gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Quick Start Protocol

For a typical $MgCl_2$ calibration for 20 µL PCRs, using a 5x PCR Master Mix, we suggest:

1. Check the current $MgCl_2$ concentration in your PCR Master Mix. A standard final concentration is 1.5 mM, or 7.5 mM in a 5x Master Mix.

2. Make a 5x dilution of 25 mM $MgCl_2$ by pipetting 20 µL into a PCR tube and adding 80 µL of PCR grade water. This will result in a working concentration of 5 mM $MgCl_2$.

3. Make a 1x Master Mix for 8 PCRs of 20 µL to accommodate $MgCl_2$ concentrations ranging from that of your PCR mix to 4.5 mM, such as according to the table below.

Reagent	**Volume for 8 rxns of 20 µL**
5x Master Mix	32 µL
PCR grade water*	9.6 µL
Primer 1 (10 µM)	3.2 µL
Primer 2 (10 µM)	3.2 µL
DNA extract	16 µL
Total volume	**64 µL**

** PCR grade water can include nuclease-free water, autoclaved molecular grade water, or DEPC-treated water.*

4. Aliquot out 8 µL of 1x Master Mix into each of 7 labelled PCR tubes. The additional 8 µL is an excess in case of pipetting errors.

5. To increase the concentration of each PCR by 0.5 mM, you will need to successively add an extra 2 µL of 5 mM $MgCl_2$, making up the volume to 12 µL with PCR grade water. A suggested gradient is shown in the table below.

Suggestion: to save on pipette tip use you can pipette 50 µL of PCR grade water into a PCR tube and use this as a working stock to avoid potential contamination of the reagent tube. Pipette the required droplet volume onto the inside of each PCR tube to avoid touching the Master Mix and avoiding back transfer of reagents into the stock. Do the same for the 5 mM $MgCl_2$. Then close each PCR tube and flick to mix, ensuring the reaction mix is at the bottom of the tube.

Final $MgCl_2$ concentration	**PCR grade water added**	**5 mM $MgCl_2$ added**
1.5 mM	12 µL	0 µL
2.0 mM	10 µL	2 µL

2.5 mM	8 µL	4 µL
3.0 mM	6 µL	6 µL
3.5 mM	4 µL	8 µL
4.0 mM	2 µL	10 µL
4.5 mM	0 µL	12 µL

6. Run PCR and gel electrophoresis according to recommended settings, and compare amplified DNA to determine the most appropriate $MgCl_2$ concentration to use for this specific PCR application. This is usually the concentration that shows the clearest bands of the expected sizes with the least non-specific amplification.