A&A BIOTECHNOLOGY
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*Manual*

# CiTi Converter MSP PCR Kit

For very high specificity PCR products, intended for research and discrimination of methylated and unmethylated cytosine.

| catalog # | size |
|---|---|
| 1080-100 | 100 reactions in 50 µl |

For research use only.

**Guarantee**

A&A Biotechnology provides guarantee on this product.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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# Description

**CiTi MSP PCR Kit** is ready to use real-time Hot Start PCR mixture. Mixture is twice concentrated and contains all the components needed to perform the real-time PCR reaction except for the template DNA and primers. It includes the optimum concentration of salt and magnesium ions, thus the only reaction conditions that should be pre-optimized is the amount of added DNA template, concentration of primers and temperature profile of the PCR.

**MSP PCR** (ang. *methylation-specific PCR*) is capable of providing very high specificity PCR products, intended for research and discrimination of methylated and unmethylated cytosine.

**CiTi HotStart DNA polymerase** is a modified, chemically blocked *Taq* DNA polymerase. This modification does not allow for extension of the primer containing a single, non-complementary nucleotide on the 3' end (Fig.1). This helps to avoid amplification of unspecific DNA fragments. The design of appropriate specific primers is simpler and allow for obtain the appropriate amplification products in the study of DNA methylation. Owing to the chemically blocking, the **CiTi HotStart DNA polymerase** is inactive at room temperature while setting PCR, which prevents unspecific extension of primes partially complementary to each other. The **CiTi HotStart DNA polymerase** is fully activated at 95 °C during the initial denaturation of template DNA within 10 min.

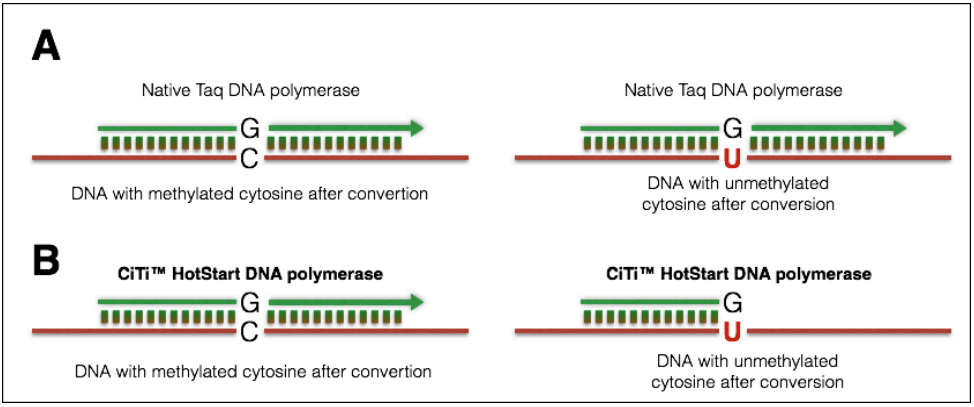


**Fig. 1. Recognition of the 3 'end mismatch of the primer by CiTi HotStart DNA polymerase.**
If the primer has guanine at the 3 'end, then annealing with a template after conversion, where unmethylated cytosine is converted to uracil results with a mismatch. Native Taq DNA polymerase extends effectively this type of structure (**A**), which leads to the formation of unspecific products. CiTi HotStart DNA polymerase because of introduced modification is unable to effectively extend the primer, where unpaired 3 'end does not match the matrix (**B**). It leads to the formation of specific PCR products only.

# Contents

| | 1080-100 | storage |
|---|---|---|
| **CiTi MSP PCR Mix** | 2 x 1,25 ml | -20 °C |
| **ultrapure water** | 2 x 1.5 ml | -20–25 °C |

## Sensitive CiTi Mix EvaGreen® composition

| component | amount |
|---|---|
| **CiTi HotStart DNA polymerase** | 0.1 U/µl |
| **$MgCl_2$** | 4 mM |
| **dNTPs** | 0.5 mM of each dNTP |
| **reaction buffer, reaction stabilizers** | |

# Additional equipment and reagents

- DNA template, primers
- vortex
- microcentrifuge
- thermocycler

## Important notes

- All solutions should be thawed thoroughly on ice, gently mixed by inverting the tube and briefly centrifuged before use.
- Up to 5x repeated freeze-thaw cycles do not influence the activity of this product.
- It is recommended to use the template DNA after conversion using the CiTi Converter DNA Methylation Kit (not included, cat. # 027-50, 027-250).

# PCR protocol example

## Before setting the reaction:

1. For setting the reaction, it is recommended to use the template DNA after conversion using A&A Biotechnology methylation kit:
    - CiTi Converter DNA Methylation Kit (cat. # 027-50, # 027-250)

2. Please note that CiTi HotStart DNA polymerase requires activation at 95 °C within 10 min, which should be taken into account in the profile of temperature-time of PCR.

3. The CiTi MSP PCR mixture contains the optimal concentration of Mg2+ ions which gives good results of DNA amplification. However, if the DNA is suspended in bufers containing EDTA (eg. TE bufer) it may be needed to supplement the Mg2+ ions by adding e.g. 25 mM MgCl2 solution to a final concentration of 2.5 mM.

4. To avoid contamination, the PCR setup and product analysis should be performed in separate places. In addition, it is recommended to use filter-containing pipette tips.

## Setting reaction with CiTi Converter MSP PCR Kit:

1. Thaw **CiTi MSP PCR Mix** and **ultrapure water** on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.

2. Place PCR tubes on ice and add:

| | PCR reaction volume |
|---|---|
| **component** | **50 µl** |
| **CiTi MSP PCR Mix** | 25 µl |
| **Primer A*** | 0.3-0.4 µM* |
| **Primer B*** | 0.3-0.4 µM* |
| **DNA template** | >3 ng |
| **ultrapure water** | up to 50 µl |

* final concentration in reaction mixture

4. Gently vortex the samples and briefly centrifuge to collect all droplets remaining on the tube walls and caps to the bottom of the tube.

5. Place the tubes in the thermocycler and start the PCR programme.

| step | temperature | time |
|---|---|---|
| **Initial denaturation** | 95 °C | 10 min |
| **35 - 45 cycles** | 95 °C<br>50-55 °C<br>72 °C | 15 s<br>30 s<br>30-60 s* |
| **final extension** | 72 °C | 5 min |
| **cooling** | 10 °C | |

* depending on the length of PCR products, for product >500 bp 1 min

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