A&A BIOTECHNOLOGY
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CiTi Converter MSP PCR Kit

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

version 0224

100 reactions in 50 µl

Cat. # 1080-100

It is recommended to use the template DNA after conversion using the CiTi Converter DNA Methylation Kit (not included, cat. # 027-50, 027-250).

Mix Contents

Component	Quantity
2x CiTi MSP PCR Mix	2 x 1250 µl
CiTi HotStart DNA polymerase $MgCl_2$ dNTPs (dATP, dCTP, dGTP, dTTP) Tris-HCl, KCl, $(NH_4)_2SO_4$ stabilizers of PCR reaction	
Sterile water (nuclease free, DEPC treated)	2 x 1500 µl

Store at -20 °C

Up to 5x repeated freeze-thaw cycles do not influence the activity of the enzyme and efficiency of DNA amplificaton.

Equipment and materials that are not included in this kit

1. DNA templates
2. Primers

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

Product is recommended for R&D use only.

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

* not adhering to the supplied protocol
* not recommended use of equipment and materials
* the use of other reagents than recommended or which are not a component of the kit
* the use of expired or improperly stored reagents

Description

MSP PCR (methylation-specific) 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 Taq polymerase. This modification does not allow for extension of the primer containing single, non-complementary nucleotide on the 3' end. This helps to avoid amplification of unspecific DNA fragments. The design of appropriate specific primers is simpler and allows for obtaining the appropriate amplification products in the study of DNA methylation. Owing to the locking with a chemically blocking the CiTi HotStart DNA polymerase is inactive at room temperature with setting PCR, which prevents unspecific extension partially complementary to each others primers. CiTi HotStart DNA polymerase is fully activated at 95 ℃ during the initial denaturation of template DNA within 10 min.

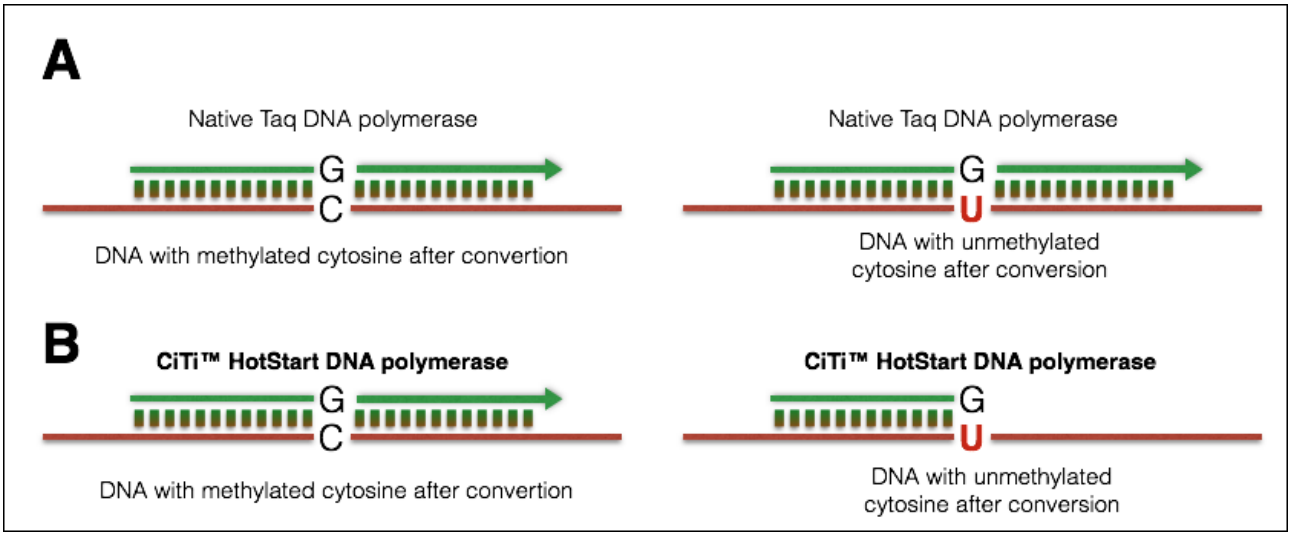

Fig. 1. Recognition of the 3 'end mismatch of the primer by CiTi HotStart DNA polymerase. If the starter is terminated at the 3 'end of guanine, the linking of the matrix after the conversion, where methylated cytosine is converted to uracil will have unpaired 3' end of the template. Native Taq DNA polymerase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

Mixture of 2x CiTi MSP PCR Mix is twice concentrated and contains all the components needed to perform the 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.

The protocol settings PCR reaction with using CiTi Converter MSP PCR Kit

Before setting the reaction:

1. It is recommended to use the template DNA after conversion using the CiTi Converter DNA Methylation Kit (not included, cat. # 027-50, 027-250).

2. Please note that CiTi HotStart DNA polymerase requires activation at 95 ℃ within 10 minutes, 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 Mg^{2+} ions which gives good results of DNA amplification. However, if the DNA is suspended in buffers containing EDTA (eg. TE buffer) it may be needed to supplement the Mg^{2+} ions by adding e.g. 25 mM $MgCl_2$ solution to a final concentration of 2.5 mM.

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

Setting reaction with CiTi Converter MSP PCR Kit:

1. Thaw 2x CiTi MSP PCR Mix, sterile water, the DNA primers and matrix.

2. Mix all solutions by vortexing, then if necessary, centrifuge briefly and aliquot into PCR tubes (Tab. 1).

component	volume
2x CiTi MSP PCR Mix	25 µl
Primer A	x µl (finished concentration 0.3-0.4 µM)
Primer B	x µl (finished concentration 0.3-0.4 µM)
DNA template	x µl (> 3 ng per reaction)
sterile water	up to 50 µl

Tab. 1. Component of reaction using CiTi Converter MSP PCR Kit.

3. If you use a thermocycler without heated lid, add about 50 µl of mineral oil to tubes in order to prevent water evaporating from the reaction mixture.

4. After mixing, the components of the reaction should be gently vortexed and centrifuged.

5. Set the thermocycler according to the manufacturer's instructions following the sample PCR profile below (Tab. 2).

step	profile	description
Initial denaturation	95 ℃ – 10 min	Activated CiTi HotStart DNA polymerase and denaturation of template DNA
The main reaction 3 – steps cycles – 35–40	95 ℃ – 15 s 50–55 ℃ – 30 s 72 ℃ – 30 s	Denaturation Anneling –temp. is about 5–8 ℃ below the Tm of the primers used Elongation – for products above 500 bp should be used elongation time 1 min
Final elongation	72 ℃ – 5 min	
Hold	10 ℃ – ∞	

Tab. 2. Thermocycling conditions for a routine CiTi Converter MSP PCR.

6. After the reaction, PCR products should be stored at 4–8 ℃ or at –20 ℃ in the case of long storage periods.

Safety information

All components from this kit are non-hazardous

Notes: