A&A BIOTECHNOLOGY
innovating life science

Manual

Sensitive CiTi Mix EvaGreen®

High specificity ready-to-use mix for real-time Hot Start PCR with EvaGreen®. Optimized for epigenetics analysis by HRM technique. 2x concentrated.

catalog #	size
2017CT-200	200 reactions in 20 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

Sensitive CiTi Mix EvaGreen® is ready to use real-time Hot Start PCR mixture optimized for high specificity, dedicated for epigenetics analysis by HRM technique. 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.

HRM PCR (ang. *high resolution melt PCR*) is a novel, homogeneous, close-tube, post-PCR method, enabling the analysis of genetic and epigenetic variations (SNPs, mutations, methylations) in PCR amplicons.

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.

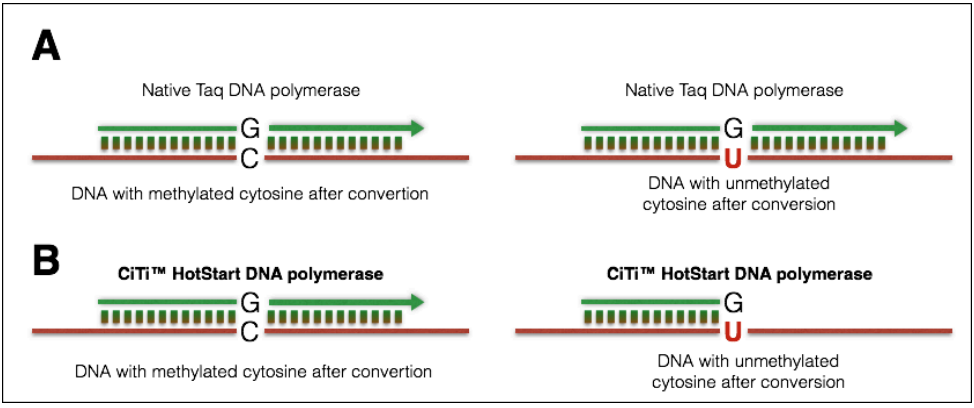

Fig. 1. Recognition of the 3 'end mismatch of the primer by CiTi HotStart DNA polymerase.
If the primer has guanine at the 3 'end, then annealing with a template after conversion, where unmethylated cytosine is converted to uracil results with a mismatch. Native Taq DNA polymerase extends effectively 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

	2017CT-200	storage
Sensitive CiTi Mix EvaGreen®	2 x 1 ml	-20 °C, in darkness
Sterile water	2 x 1.5 ml	-20 °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
2x reaction buffer with EvaGreen®	

Additional equipment and reagents

- MagnifiQ™ 16 CiTi Converter DNA Methylation instant kit (cat. # 027A-16U-64); MagnifiQ™ CiTi Converter DNA Methylation kit (cat. # 027MB-50); CiTi Converter DNA Methylation Kit (cat. # 027-50)
- 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 3x repeated freeze-thaw cycles do not influence the activity of this product.

ROX reference dye

Some PCR instruments perform fluorescence signal correction and it is recommended to use ROX reference dye for signal normalization. Please follow manufacturer's instructions regarding addition of ROX reference dye and its concentration.

PCR protocol example

1. For setting the reaction, it is recommended to use the template DNA after conversion using A&A Biotechnology methylation kit:
   - MagnifiQ™ 16 CiTi Converter DNA Methylation instant kit (cat. # 027A-16U-64)
   - MagnifiQ™ CiTi Converter DNA Methylation kit (cat. # 027MB-50)
   - CiTi Converter DNA Methylation Kit (cat. # 027-50)

2. Thaw **Sensitive CiTi Mix EvaGreen®** and **sterile water** on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.

3. Place PCR tubes on ice and add:

	PCR reaction volume	
component	**10 µl**	**20 µl**
Sensitive CiTi Mix EvaGreen®	5 µl	10 µl
Primer 1**	0.1-1 µM*	0.1-1 µM*
Primer 2**	0.1-1 µM*	0.1-1 µM*
DNA template	3 ng-1 µg	3 ng-1 µg
ultrapure water	up to 10 µl	up to 20 µl

* recommended for standard real-time PCR
** 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.

   An example amplification profile:

step	temperature	time
Initial denaturation	95 °C	10 min
35 - 45 cycles	95 °C 50-68 °C 72 °C	15-30 s 30-60 s 15-60 s*

* depending on the length of PCR products, for product >500 bp 1 min
PCR product melting analysis is recommended.

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