A&A BIOTECHNOLOGY
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Manual

MagnifiQ™ CiTi Converter DNA Methylation Kit

Complete kit for efficient conversion and preparation of converted DNA for methylation research. Purification method using magnetic beads.

catalog #	size
027MB-50	50 reactions
027MB-250	250 reactions

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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Advantages

- High-throughput, complete conversion of GC-rich DNA.
- Minimized DNA degradation and loss during treatment.
- Simple and efficient magnetic purification method.
- Eluted, ultra-pure DNA is ideal for use in molecular analyses.

Description

MagnifiQ™ CiTi Converter DNA Methylation kit is designed for bisulfite conversion and purification of converted DNA for high-throughput methylation analysis. Purification method uses magnetic beads and a magnetic stand dedicated for manual operation. The kit has been designed to provide complete conversion of unmethylated cytosines and to minimize template degradation and loss of DNA during treatment and clean-up.

Contents

	027-50MB		027-250MB		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
C/T conversion reagent	5 pcs	K-C/T-1	25 pcs	K-C/T-1	15–25 °C
D dilution solution	1.5 ml	K-D-15A	8 ml	K-D-8	15–25 °C
MQBB magnetic beads	1.1 ml	K-MQBB-11A	5.5 ml	K-MQBB-55A	15–25 °C
G binding solution	35 ml	K-G-35	165 ml	K-G-165	15–25 °C
A1 wash solution	30 ml	K-A1-30	150 ml	K-A1-150	15–25 °C
DS desulphonation solution	12 ml	K-DS-12	60 ml	K-DS-60	15–25 °C
Tris buffer	3 ml	K-TRIS-3	15 ml	K-TRIS-15	15–25 °C
ultrapure water	15 ml	K-WUP-15	50 ml	K-WUP-50	-20–25 °C

Additional equipment and reagents

- magnetic stand
- 1.5 - 2 ml Eppendorf tubes
- vortex
- microcentrifuge
- thermoblock

Important notes

- **C/T** conversion reagent is supplied as a solid crystalline at an amber tube and it is light sensitive! For best results, **C/T** conversion reagent should be used immediately following preparation.

Preparation of C/T conversion reagent

1. Add **750 µl** of ultrapure water and **210 µl** of **D** dilution solution to the tubes with **C/T** conversion reagent.
2. Mix by vortexing or shaking for **10 min** at r**oom temp**.

   Note. Each tube of C/T conversion reagent is designed for 10 separate DNA treatments.

   Note. C/T conversion reagent solution can be stored: overnight at room temp., up to one week at 4 °C, up to one month at -20 °C.

Protocol of DNA conversion

The conversion reaction can process a sample containing **500 pg-2 µg** of DNA. For optimal results, the amount of input DNA should be contained within a range of **200-500 ng**.

1. Add ultrapure water to DNA samples up to a total volume of **50 µl**.
   Add **100 µl** of **C/T** conversion reagent to each sample and mix by pipetting.

   Attention. No vortexing.
2. Incubate the samples in the dark for **10 min** at **98 °C** and then **2.5 h** at **64 °C.**
3. Incubate samples for **10 min** on ice (**0-4 °C**)**.**

   Note. Samples can be stored at 4 °C for up to 20 hours.

Protocol of purification of converted DNA

1.	Add **600 µl** of **G** binding solution and **20 µl** of **MQBB** magnetic beads to each tube. Mix by pipetting and incubate at **room temperature** for **5 min**.
2.	Spin the tubes briefly and transfer to a magnetic stand. Remove the supernatant carefully.
3.	Remove the tubes from the magnetic stand for this and each subsequent buffer addition.
4.	Add **100 µl** of **A1** wash solution. Mix by pipetting.
5.	Spin the tubes briefly and transfer to a magnetic stand. Remove the supernatant carefully.
6.	Add **200 µl** of **DS** desulphonation solution. Mix by pipetting and incubate at **room temperature** for **10 min**.
7.	Spin the tubes briefly and transfer to a magnetic stand. Remove the supernatant carefully.
8.	Add **200 µl** of A**1** wash solution. Mix by pipetting.
9.	Spin the tubes briefly and transfer to a magnetic stand. Remove the supernatant carefully.
10.	Again add **200 µl** of **A1** wash solution. Mix by pipetting.
11.	Spin the tubes briefly and transfer to a magnetic stand. Remove the supernatant carefully.
12.	Dry the tubes with lids open for **10 min** at **room temperature**.
13.	Add **15-40 µl** of **Tris buffer** or **ultrapure water**. Mix by pipetting. Incubate at **65 °C** for **10 min**.
14.	Spin the tubes briefly and transfer to a magnetic stand. Transfer DNA eluates to new sterile tubes. **Note.** The DNA is ready for immediate analysis or can be stored at or below -20 °C for later use.

Safety information

DANGER

A1 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

WARNING

C/T conversion reagent

H302 Harmful if swallowed.
H319 Causes eye irritation.
P264 Wash skin thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P312 If swallowed: Call a Poison Center/doctor if you feel unwell.
P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 If eye irritation persists: Get medical advice/ attention.

DANGER

D dilution solution

H290 Corrosive to metals.
H314 Causes serious eye irritation.
P260 Do not breathe dust.
P280 Wear protective gloves / protective clothing / eye protection / face protection.
P303+P361+P353 If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304+P340+P310 If inhaled: Remove person to fresh air and keep comfortable for breathing. Immediately call a Poison Center / doctor.
P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor / physician.

DANGER

DS desulphonation solution

H225 Highly flammable liquid and vapour.
H290 Corrosive to metals.
H314+H319 Causes serious eye and skin irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P261 Avoid breathing vapours.
P280 Wear protective gloves / protective clothing / eye protection / face protection.
P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor / physician.

WARNING

G binding solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

A&A BIOTECHNOLOGY
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A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 883 323 761, +48 600 776 268
info@aabiot.com, www.aabiot.com

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