A&A BIOTECHNOLOGY
innovating life science

Manual

Fast CiTi Converter DNA Methylation Kit

Complete kit for efficient conversion and purification of converted DNA for methylation research. Contains ready to use Fast C/T reagent developed for time-saving and convenient conversion process.

catalog #	size
027F-50	50 reactions
027F-200	200 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

Table of Contents

Advantages

- High-throughput, complete conversion of GC-rich DNA.
- DNA conversion within 60 minutes.
- Liquid, ready to use conversion reagent.
- Eluted, ultra-pure DNA is ideal for use in molecular analyses.

Description

Fast CiTi Converter DNA Methylation Kit includes a set of reagents for bisulfite conversion and purification of converted DNA for high-throughput methylation analysis. The kits have been designed to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Fast C/T conversion reagent, on the contrary to conventional bisulfite reagent, is a ready-to-use solution and performs complete conversion within 60 minutes.

Contents

	027F-50		**027F-200**		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
microcolumns with 2 ml tubes	50 pcs	K-CT-50	200 pcs	K-CT-200	15–25 °C
Fast C/T conversion reagent	5 x 1.1 ml	K-FC/T-11A	20 x 1.1 ml	K-FC/T-11A	15–25 °C
G binding solution	35 ml	K-G-35	130 ml	K-G-130	15–25 °C
A1 wash solution	30 ml	K-A1-30	110 ml	K-A1-110	15–25 °C
DS desulphonation solution	12 ml	K-DS-12	45 ml	K-DS-45	15–25 °C
Tris buffer	2 ml	K-TRIS-2	8 ml	K-TRIS-8	15–25 °C
ultrapure water	8 ml	K-WUP-8	30 ml	K-WUP-30	-20–25 °C

Additional equipment and reagents

- 1.5 ml sterile Eppendorf tubes
- vortex
- microcentrifuge
- thermal cycler

Important notes

- **Fast C/T** conversion reagent is supplied as a solution in amber tube and it is light and oxygen sensitive. For best results, one tube should be completely used up before the next one is opened.
- Using a thermal cycler is highly recommended to ensure uniform heating and to avoid evaporation of samples.

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 within a range of **200-500 ng**.

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

   Attention. No vortexing.

2. Incubate the samples in the thermal cycler for **2 min at 98°C** and then **60 min at 85 °C.**

3. Cool down samples 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 to each tube.
Mix by inverting the tubes.

2. Transfer the mixtures **into the microcolumns**.
Close the tubes with the caps.

3. Centrifuge for **30-60 s** at **10 000-15 000 RPM** (≥10 000 x g).

4. Remove the microcolumns from the tubes and discard flow-through.
Place back the microcolumns into **the same** tubes.

5. Apply onto the microcolumns **100 µl** of **A1** wash solution.
Close the tubes with the caps.

6. Centrifuge for **30-60 s** at **10 000-15 000 RPM** (≥10 000 x g).

7. Apply onto the microcolumns **200 µl** of **DS** desulphonation solution.
Close the tubes with the caps. Incubate for **10 min** at **room temp.**

8. Centrifuge for **30-60 s** at **10 000-15 000 RPM** (≥10 000 x g).

9. Remove the microcolumns from the tubes and discard flow-through.
Place back the microcolumns into **the same** tubes.

10. Apply onto the microcolumns **200 µl** of **A1** wash solution.
Close the tubes with the caps.

11. Centrifuge for **30-60 s** at **10 000-15 000 RPM** (≥10 000 x g).

12. Apply onto the microcolumns **200 µl** of **A1** wash solution.
Close the tubes with the caps.

13. Centrifuge for **2 min** at 10 **000-15 000 RPM** (≥10 000 x g).

14. **Attention.** While adding the elution buffer into the microcolumn ensure that liquid is being applied precisely onto the resin. If some of the liquid stays on the column walls the elution may not be effective.

	Transfer dried microcolumns into sterile **1.5 ml** elution tubes (not included). Add precisely **15-30 µl** of **Tris** buffer or ultrapure water onto the microcolumns resin. Close the tubes with the caps. **Note.** Elution volume of 30 µl is recommended for fragments of size exceeding 2000 bp.
15	Incubate the microcolumns for **3 min** at **room temp**.
16.	Centrifuge for **1 min** at **10 000-15 000 RPM** (≥10 000 x g).
17.	Remove microcolumns, close the elution tubes. **Note.** Store the purified DNA samples at +4 °C to +8 °C.

Frequently Asked Questions

Question: What amount of DNA is needed for efficient conversion of DNA?
Answer: The conversion reaction can be processed as a sample containing 500 pg-2 µg of DNA. For optimal results, the amount of input DNA should be within a range of 200-500 ng.

Question: What is the chemical conversion efficiency of DNA by using CiTi Converter Methylation Kit?
Answer: More than 99% of non-methylated C residues are converted to U; with consistent > 99% protection of methylated cytosines.

Question: What is the efficiency of DNA purification upon conversion reaction?
Answer: Average yield of about 80%.

Question: Which polymerase(s) do you recommend for PCR amplification of converted DNA?

Answer: The following A&A Biotechnology product is recommended: **Sensitive CiTi Mix EvaGreen®** (cat. # 2017CT-200).

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

Fast 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

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

innovating life science

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