GENE CKER
For Genetic Diagnosis & Testing

User Manual

Instructions for Use

CRISmono™ DNMT3A

Please read all contents in the product manual before use.

Research Use Only. Not for use in diagnostic procedures.

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1. Product Information

Important: Before using this product, read and understand the information in the "**B. Precautions for Use**" of the **Appendix** in this document.

1.1. Product Description

CRISmono™ DNMT3A is a Sanger sequencing kit designed to detect mutations in genomic DNA (gDNA) from the blood for cancer risk assessment including hematological malignancies. Utilizing GeneCker's ultra-precision CRISPR-Cas9 system, this kit effectively detects even low-frequency mutations by selectively cutting wild-type DNA and amplifying mutant DNA. This product is designed to analyze the DNMT3A gene R882 hotspot mutation, a representative mutation in hematological malignancies. Results from this kit are intended for clinical reference only. Clinicians should comprehensively interpret the test results in the context of the examinee's condition, drug indications, treatment plan, and other laboratory test indicators.

1.2. Product Component and Storage Condition

Important: Upon arrival, inspect all consumables and contact Warranty Service team if any of the components have been damaged during shipping, store all components under the recommended condition and in an upright position.

Category	Kit Components	Cap Color	Quantity	Volume (µL)	Storage
***In vitro* Cleavage**	● DNMT3A Enzyme mix	Blue	1	100 µL	-20℃
	● Remov RXN buffer	Red	1	100 µL	
	● Stabilizer	Red	1	25 µL	
	● 10X STOP buffer	Red	1	25 µL	
Target Amplification	○ 2X PCR Master mix	White	1	625 µL	
	● DNMT3A primer mix	Blue	1	125 µL	
Sanger Sequencing	● DNMT3A Seq primer F (10 pmole/µL)	Purple	1	25 µL	

1.3. Required Equipment and Material (Not Provided)

Besides the components of the kit, the following equipment and materials are also required.

1) List of equipment and consumables

	Equipment	Supplier / Catalog No.
1	96-well Thermal Cycler	General laboratory supplier
2	Microcentrifuge	General laboratory supplier
3	Vortex Mixer	General laboratory supplier
4	Cooling Rack	General laboratory supplier

2) List of materials

	Other Materials	Supplier / Catalog No.
1	Microtubes, 1.5 mL	General laboratory supplier
2	PCR Tube	General laboratory supplier
3	DEPC-Water	General laboratory supplier
4	PCR product purification kit	General laboratory supplier

2. Testing Protocol

2.1. Important Notes

1) All reagents used in this protocol should be performed briefly spin down to collect components clinging under the lid and wall of tubes.
2) For frozen reagents, they are fully thawed and mixed using a vortex mixer. Briefly spin down.
3) Keep all reagents on ice until ready for use.
4) If all reagents are repeatedly frozen and thawed, it may lead to deteriorated performance.
5) The use of filter tips throughout the entire process is recommended.

2.2. Time Required for 25 Samples

Part	Step	Time (hrs)
***In vitro* Cleavage**	*In vitro cleavage* (*IVC*)	1.5
Target Amplification	PCR	2
	PCR product purification	0.5
Sanger Sequencing	Sanger sequencing	-

2.3. Sample Preparation

1) Sample preparation

- This product uses DNA that meets the summary of recommended DNA quality and quantity standards below.

2) Summary of recommended DNA quality and quantity

권장하는 DNA 품질		
gDNA	Amount of Input DNA	20~150 ng
	DNA Quality (OD A260/280 Ratio)	1.8~2.0

2.4. *In vitro* Cleavage (IVC)

Materials (Including Kit)
- 🔵 DNMT3A Enzyme mix - 🔴 Remov RXN buffer - 🔴 Stabilizer - 🔴 10X STOP buffer
User preparing materials
- Thermal Cycler - 1.5 mL Microtube - PCR Tube - DEPC-Water

1) Thaw the 🔵 DNMT3A Enzyme mix, 🔴 Remov RXN buffer, 🔴 Stabilizer on ice using a cooling rack, and then vortex and spin down.
2) Add the DNA sample and each reagent to the 8-strip tube under the conditions shown in the table below. When handling multiple samples, it is recommended to prepare and use a master mix in a 1.5 mL microtube.

	Reagent	Volume per Sample (μL)
***In vitro* Cleavage (IVC)**	🔴 Remov RXN buffer	4
	🔴 Stabilizer	1
	🔵 DNMT3A Enzyme mix	4
	gDNA	1
	Total Volume	**10**

3) Mix each reagent, then perform vortexing and spin down.

4) Place the 8-strip tube in the thermal cycler pre-set under the conditions shown in the table below and run the program. (Lid temperature: 60℃)

Step Description	Temperature	Time
In vitro Cleavage (IVC)	45℃	60 min

5) After the 60 minutes incubation, add 1 μL of 🔴 10X STOP buffer to IVC product 10 μL.

6) Vortex and spin down.

2.5. Target Amplification

Kit included materials
- ○ 2X PCR Master mix
- 🔵 DNMT3A primer mix
User preparing materials
- PCR tube
- DEPC-Water
- Thermal Cycler

1) Thaw the ○ 2X PCR Master mix and 🔵 DNMT3A primer mix on ice using a cooling rack, and then vortex and spin down.

2) Add the IVC product and each reagent to the 8-strip tube under the conditions shown in the table below. When handling multiple samples, it is recommended to prepare and use a master mix in a 1.5 mL microtube.

Reagent	Volume per Sample (μL)
IVC Product	2
○ 2X PCR mix	25
DEPC-Water	18
🔵 DNMT3A primer mix	5
Total Volume	**50**

3) Mix each reagent, then perform vortexing and spin down.

4) Place the 8-strip tube in the thermal cycler preset under the conditions shown in the table below and run the program. (Lid temperature: 105℃)

Step Description	Temperature	Time	Cycle
Pre-denaturation	98	3 min	1
Denaturation	98	10 sec	42
Annealing	55	40 sec	
Extension	72	30 sec	
Final Extension	72	5 sec	
Hold	4	∞	1

2.6. Sanger Sequencing (Refer to manufacturer's guidelines)

Step 1. PCR product purification

1) PCR products are purified using a commercially available PCR purification kit and then used for subsequent analysis.

- Column purification, bead purification, and enzyme purification can all be used.

Step 2. Sanger sequencing

Materials (Including Kit)
- ● DNMT3A Seq primer F (10 pmole/μL)
User preparing materials
- DNA sequencer

1) Perform Sanger sequencing using DNMT3A Seq primer F included in the product.

2.7. Data analysis

- Sequencing chromatogram analysis for DNMT3A codon 882(CDS 2644-2645) sites.
  - ■ Codon 882 - TATACTGACGTCTCCAACATGAGC**CG**C**T**TGGCGAGGCAGAGACTGCTGGGC

- If the Enriched VAF (%) exceeds 10%, it is considered a reliable value.
  - ■ $Enrichied\ VAF\ (\%) = 100 \times \frac{Mutant\ height}{(Wildtype\ height + Mutant\ height)}$
  - ■ If the Enriched VAF is less than 10%, it is recommended to re-verify by comparing it with Control (Non-IVC).

Warranty and Liability

GeneCker Co., Ltd. is not responsible for problems caused by using test methods other than the one suggested in this product manual. In the event of a problem with the product, the customer can report the problem within 30 days to GeneCker Co., Ltd. Customer Center.

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3. Appendix

A. Troubleshooting Guide

Troubles	Probable Cause	Resolution
In case the VAF (Variant Allele Frequency) doesn't change value is low or not after the *In vitro* Cleavage reaction	**Short Reaction Time**: If the reaction time for *in vitro* Cleavage is too short, there may be limited cleavage of wild-type DNA, resulting in difficulty obtaining enough of the desired target genes	Follow the manufacturer's recommended *in vitro* Cleavage conditions and consider re-reaction if necessary.
	Excessive Reaction Time: If the reaction time for *in vitro* Cleavage exceeds the manufacturer's recommended duration, excessive cleavage of the amplicon may occur.	
	In case of amplification inhibition due to excessive addition of ●10X STOP buffer after in vitro cleavage reaction	Salts may form in ●10X STOP buffer. Incubate at 37°C for 10 minutes before use.
	If the activity of the DNMT3A enzyme mixture is reduced, the cleavage efficiency of the WT gene is lowered, making it difficult to obtain the desired target gene.	The DNMT3A enzyme mixture may lose enzyme activity if frozen and thawed repeatedly, so be sure to use it in aliquots when using it multiple times.
In case the enriched VAF analysis of sequencing chromatograms is difficult	If the sequencing is not successful due to insufficient amplification of the target site	If the concentration of the sample is insufficient, the target sequence may not be amplified, so please proceed with the experiment using a sample of the recommended concentration.
		If it is difficult to perform the experiment due to low sample concentration, increase the input IVC product volume to 4 µL and perform PCR again.
	When analysis is difficult due to increased background signal caused by amplification of non-specific targets	If the next step is not performed immediately after IVC, DNA degradation may occur, which can hinder amplification. Therefore, proceed to the PCR process immediately after IVC

B. Precautions for Use

1. This product may not be used for purposes other than research.
2. This product is disposable and cannot be reused.
3. It is recommended to read the following precautions before using this product.
4. This product is designed to use Sanger sequencing, which are base sequencing analyzers.
5. Prepare a master mix of all reagents on ice.
6. It is recommended to use filter tips to prevent cross-contamination.
7. Since the quality of the DNA sample affects the results, it is recommended to use high-quality DNA. Severely damaged DNA can cause the failure of library construction.
8. Contamination by DNA and RNA other than the sample may affect the quantification of DNA extracted from the sample, so keep the surrounding environment clean to avoid cross-contamination.
9. Contamination of test reagents and equipment, reaction temperatures, or storage conditions different from those recommended may affect test results.
10. If you need help with GeneCker's product, please contact the GeneCker Co., Ltd. Support at **info@genecker.com**.

4. Documentation and Support

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, **GeneCker Co., Ltd.** WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Symbol Descriptions

The following table describes the symbols on the shipment packaging, consumable, or consumable packaging.

Symbol	Description	Symbol	Description
	Indicates the manufacturer.		Storage temperature ranges in degrees Celsius. Store the consumables within the indicated range.
	Date of manufacture.		Caution.
	Indicates the part number so that the consumable can be identified.		The date the consumable expires. For best results, use the consumable before this date.
	Indicates the batch code to identify the manufacturing batch or a lot of the consumables.		Contains sufficient for <n> tests.
	Consult instructions for use.		Do not reuse.
	The intended use is Research Use Only (RUO).		

Revision History

Document Number	Revision	The date of revision	Contents of revision
GC-IFU-A105R-EN	0	02 Oct 2024	Initial release

Customer and Technical support :)

For Customer and technical assistance, contact GeneCker Support.

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