Cat. # 6088

For Research Use

TaKaRa

TaKaRa PCR Carryover Prevention Kit

Product Manual

v1201Da

Table of Contents

I. Description

Since PCR is a highly sensitive technique, carryover of amplification products from previous PCR reactions may lead to false positive results. This is a very significant issue during endpoint PCR, particularly when testing food and environmental samples.

The TaKaRa PCR Carryover Prevention Kit is designed to preclude false-positive results caused by carryover contamination. This product includes dUTP instead of dTTP for PCR. It also includes UNG (uracil-*N*-glycosylase), an enzyme that degrades uracil-containing DNA. Treatment with UNG prior to PCR degrades uracil-containing PCR contaminants but leaves thymine-containing templates intact, allowing selective removal of carryover PCR products.

Degradation by UNG takes place in the following manner. In a pre-PCR reaction at 25℃ for 10 min, UNG hydrolyzes *N*-glycosylic bonds between the deoxyribose sugars and the uracil bases in uracil-containing DNA, leaving apyrimidinic sites in the DNA. Next, a heat treatment at 95℃ for 2 min deactivates UNG and simultaneously causes cleavage and degradation of the contaminant DNA fragments by hydrolysis of the phosphate backbone at the abasic sites.

UNG hydrolyzes uracil-containing single- and double-strand DNAs but shows no activity toward RNA. This kit includes a dUTP-containing dNTP mixture and an $MgCl_2$ solution in addition to UNG. Use this kit in combination with *TaKaRa Taq* Hot Start Version (Cat. #R007A/B) or other Pol I-type PCR enzymes.

II. Kit Components (for 200 reactions, 50 μl volume)

(1)	UNG	2 U/μl	100 μl
(2)	dU plus dNTP Mixture*	12.5X	800 μl
(3)	$MgCl_2$	25 mM	1 ml

* : dU plus dNTP Mixture is an aqueous solution (sodium salt) with the following composition:

dUTP	7.5 mM
dATP	2.5 mM
dGTP	2.5 mM
dCTP	2.5 mM

III. Storage -20℃

IV. Materials Required but not Provided

PCR enzyme

- *TaKaRa Taq* Hot Start Version (Cat. #R007A/B), *TaKaRa Taq* (Cat. #R001A/B), or other Pol I-type PCR enzymes

  Note: α -Type enzymes with proofreading activity bind to uracil-containing templates and may inhibit PCR reactions. Takara Bio does not recommend the use of this product in combination with an α-type PCR enzyme or an enzyme blend containing an α-type PCR enzyme.

Micropipettes

Thermal Cycler

Tips for micropipettes

V. Precautions before Use

Designate and physically isolate the four laboratory areas described below for performing the indicated processes.

- ○ Area 1: Reaction mixture preparation and dispensing
- ○ Area 2: Sample preparation
- ○ Area 3: Addition of samples to reaction mixtures
- ○ Area 4: Reactions and electrophoretic detection

Do not open/close tubes containing amplification products in any of the areas except Area 4. Doing so may cause contamination.

VI. Protocol

When using *TaKaRa Taq* Hot Start Version (Cat. #R007A/B)

1. Prepare the reaction mixture below on ice. (Work in Area 1).
   Prepare master mixes (without template) in volumes sufficient for the required number of tubes plus a few extra. Dispense into reaction tubes and cap loosely.

TaKaRa Taq Hot Start Version (5 units/μl)	0.25 μl
10X PCR Buffer (Mg^{2+} plus)＊1	5 μl
dU plus dNTP Mixture＊2	4 μl
$MgCl_2$＊2,3	1.5 μl
UNG＊2,4	0.5 μl
Template	< 500 ng
Primer 1	10 - 50 pmol (final conc. 0.2 - 1.0 μM)
Primer 2	10 - 50 pmol (final conc. 0.2 - 1.0 μM)
Sterile distilled water	to 50 μl

   ＊1： Supplied with *TaKaRa Taq* Hot Start Version (Cat. #R007A/B)
   ＊2： Supplied with this kit
   ＊3： Since the dUTP at a concentration is 3 times that of dTTP, the total concentration of dNTPs is high overall. Although the PCR Buffer contains $MgCl_2$, it is still necessary to add more $MgCl_2$ in order to maintain a proper balance between the amounts of $MgCl_2$ and dNTP. Add 4.5 μl when using 10X PCR Buffer (Mg^{2+} free).
   ＊4： The standard amount to use is 1 U per 50 μl reaction.

2. Add the sample (template). (Work in Area 3).
   Add the sample to the reaction mixture prepared in step 1 and cap tightly.
3. Briefly centrifuge the tubes in a microcentrifuge for 0.2-ml tubes and set them in a thermal cycler.
4. Perform UNG treatment and PCR amplification.
   Perform a UNG treatment first, followed by a UNG heat inactivation. Next, perform an amplification reaction under the normal PCR conditions. Optimize PCR conditions according to the amplification product size and other considerations as described.

   <Example: Amplification of 1 kb DNA product>

25℃	10 min.	(UNG treatment)＊1	
95℃	2 min.	(UNG heat inactivation)＊1	
98℃	10 sec.	＊2	30 cycles
55℃	30 sec.		
72℃	1 min.	＊3	

   ＊1： The UNG treatment conditions remain the same, regardless of the size of the amplification product.
   ＊2： Choose the PCR denaturation conditions according to the types of thermal cycler and reaction tube used. In general, use 98℃ for 5 - 10 sec. or 94℃ for 20 - 30 sec.
   ＊3： The amplification efficiency may be slightly decreased due to use of dUTP instead of dTTP. Increase the extension step time if the amplification efficiency is low.
5. Analyze the PCR reaction mixture by electrophoresis or other assays. (Work in Area 4)

VII. Experimental Example

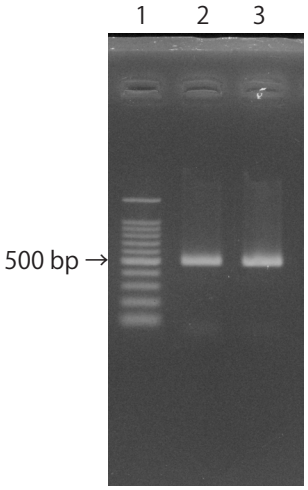
1. Comparison of PCR amplification efficiency using the TaKaRa PCR Carryover Prevention Kit versus conventional PCR

[Method] A comparison of amplification efficiency was performed between a reaction with conventional PCR composition and a reaction where PCR was performed using *TaKaRa Taq* Hot Start Version and the TaKaRa PCR Carryover Prevention Kit. Using 50 ng of human genomic DNA as template, an approximately 500 bp DNA was amplified.

<Conventional PCR reaction>
- PCR conditions were as recommended for *TaKaRa Taq* Hot Start Version
- PCR performed without UNG, but with the dTTP containing dNTP mixture

<PCR reaction with UNG>
- PCR conditions were as recommended for this product
- PCR performed with both UNG and the dUTP-containing dU plus dNTP Mixture

[Results] The results confirmed that PCR with UNG achieved excellent amplification, equivalent in efficiency to conventional PCR.

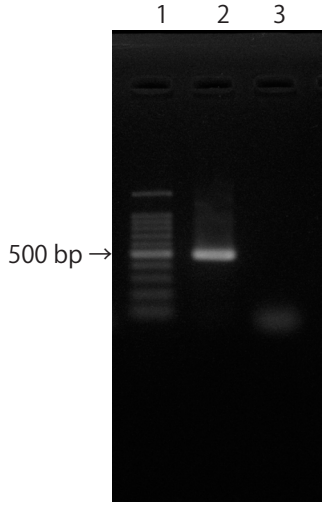


1. 100 bp DNA Ladder
2. PCR product prepared using conventional PCR
3. PCR product prepared using TaKaRa PCR Carryover Prevention Kit and *TaKaRa Taq* Hot Start Version

2. Suppression of amplification product carryover

[Method] Using 10 ng of human genomic DNA as template, an approximately 500 bp PCR product was amplified according to the protocol for this kit (1st PCR). Using 2 μl of the PCR amplification product as template, a PCR amplification was carried out in the same manner as in the 1st PCR with or without UNG treatment (2nd PCR) to verify whether carryover prevention occurred.

[Results] In the absence of UNG treatment, the 2nd PCR produced a PCR amplification product by using the 1st PCR product as template. With UNG, the 2nd PCR did not yield product, confirming efficient suppression of amplified product originating from carryover template.



1. 100 bp DNA Ladder
2. 2nd PCR product without UNG treatment
3. 2nd PCR product with UNG treatment

VIII. Related Products

Uracil DNA Glycosylase (UNG), heat-labile (Cat. #2820)
dU plus dNTP Mixture (12.5X) (Cat. #4035)
dUTP (Cat. #4020)
TaKaRa Taq™ (Cat. #R001A/B)
TaKaRa Taq™ Hot Start Version (Cat. #R007A/B)
TaKaRa PCR Thermal Cycler Dice™ Gradient/Standard (Cat. #TP600/TP650)*
Mupid®-2plus (Cat. #M-2P)
Mupid®-exU (Cat. #EXU-1)
20X TAE Buffer (Cat. #28354)
TBE (Tris-borate-EDTA) powder (Cat. #T905)
100 bp DNA Ladder (Cat. #3407A/B)

* Not available in all geographic locations. Check for availability in your region.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC.

If you require licenses for other use, please contact us by phone at +81 77 543 7247 or from our website at www.takara-bio.com.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product web page. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.