Cat. # R013S/A

For Research Use

TaKaRa

TaKaRa Taq™ HS PCR Kit, UNG plus

Product Manual

v202101Da

Table of Contents

I. Description

PCR is a highly sensitive detection technique. Any carryover of amplification products from a previous PCR may lead to false positive results. The potential for such false positives is high, particularly with some food and environmental sample testing involving repeated rounds of PCR. *TaKaRa Taq* HS PCR Kit, UNG plus prevents false positive results caused by carryover of amplicons.
The kit allows amplification using *TaKaRa Taq* HS, a hot-start PCR enzyme, with dUTP in place of dTTP as the substrate. PCR reaction mixtures are treated with uracil-*N*-glycosylase (UNG), an enzyme that cleaves uracil-containing DNA. UNG degrades uracil-containing DNA derived from a previous PCR while leaving non-uracil-containing templates unaffected, allowing PCR amplifications to take place with templates while suppressing amplification of carryover 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 DNA but has no effect on RNA.

II. Components

R013S (for 50 reactions, 50 μl volume)

1.	*TaKaRa Taq* HS	5 U/μl	12.5 μl
2.	10X PCR Buffer for UNG plus＊1		250 μl
3.	dU plus dNTP Mixture＊2	12.5X	200 μl
4.	UNG	2 U/μl	25 μl

R013A (for 200 reactions, 50 μl volume)

1.	*TaKaRa Taq* HS	5 U/μl	50 μl
2.	10X PCR Buffer for UNG plus＊1		1 ml
3.	dU plus dNTP Mixture＊2	12.5X	800 μl
4.	UNG	2 U/μl	100 μl

＊1 Mg^{2+} concentration: 22.5 mM (10X)
Since the dUTP concentration is 3 times that of dTTP, the total concentration of dNTPs is high overall. In order to maintain balance between levels of $MgCl_2$ and dNTP, this kit contains a higher concentration of Mg^{2+} compared with that of 10X PCR Buffer (Mg^{2+} plus) supplied with *TaKaRa Taq* Hot Start Version (Cat. #R007A).

＊2 dU plus dNTP Mixture is an aqueous solution (sodium salt) containing the following components:

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

Micropipettes
Tips for micropipettes
Thermal cycler
Tubes for PCR

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 DNA contamination.

VI. Protocol

1. Prepare the reaction mixture on ice. (Work in Area 1).
   Prepare a master mixture of all components except the template (e.g., sample) in a volume sufficient for the required number of PCR reactions plus a few extra. Dispense the mixture into reaction tubes and cap loosely.

Reagent	Amount	Final conc.
TaKaRa Taq HS (5 U μl)	0.25 μl	
10X PCR Buffer for UNG plus	5 μl	
dU plus dNTP Mixture	4 μl	
UNG	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 purified water	Up to 50 μl	

2. Add samples (template). (Work in Area 3)
   Add samples (template) to reaction mixtures dispensed in step 1 and cap tightly.

3. Spin down briefly in a tabletop centrifuge. Then set the tubes in a thermal cycler.

4. Perform UNG treatment and PCR amplification.
   First, carry out UNG treatment, followed by heat inactivation of UNG. Next, perform an amplification reaction under standard PCR conditions. Select PCR conditions suitable for the amplification size and other factors.

   (Example) PCR Amplification of 1 kb DNA

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

   ＊1 The UNG treatment conditions remain the same, regardless of the size of the amplification product.

   ＊2 Perform PCR denaturation under conditions suitable for the thermal cycler and reaction tube used. In general, denature at 98℃ for 5 to 10 sec or at 94℃ for 20 to 30 sec.

   ＊3 Since dUTP is used in place of dTTP, the amplification efficiency may decrease slightly. In case of poor amplification efficiency, prolong the extension time.

5. Analyze the PCR reaction mixture by electrophoresis or other analytical techniques. (Work in Area 4)

VII. Experimental Examples

1. Comparison of PCR amplification efficiency using this 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 the *TaKaRa Taq* HS PCR Kit, UNG plus. Fifty ng of human genomic DNA was used as template. The amplification product was approximately 500 bp.

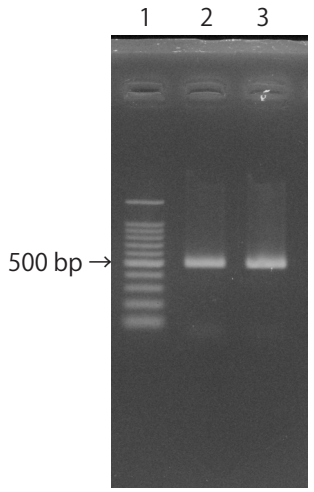
<Conventional PCR reaction>

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

<PCR reaction with UNG>

- PCR conditions were as recommended for this kit
- Reaction performed with both UNG and the dU plus dNTP Mixture

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

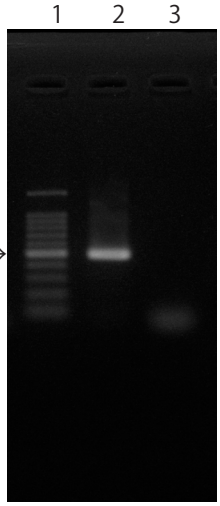


1. 100 bp DNA Ladder
2. PCR products using the conventional method
3. PCR products using UNG

2. Suppression of amplification product carryover

   [Method] In the first PCR, amplification of an approximately 500 bp product was conducted using 10 ng of human genomic DNA as template in accordance with the protocol for *TaKaRa Taq* HS PCR Kit, UNG plus. To verify the suppression of carryover products, a subsequent PCR amplification was carried out using 2 μl of the PCR product from the first PCR as template, with and without UNG treatment (second PCR).

   [Result] The second PCR without UNG treatment resulted in PCR amplification with the product from the first PCR as template. The second PCR with UNG treatment, on the other hand, resulted in no PCR amplification, demonstrating the inhibition of carryover.



1. 100 bp DNA Ladder
2. 2nd PCR products without UNG treatment
3. 2nd PCR products 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 PCR Carryover Prevention Kit (Cat. #6088)＊
TaKaRa Taq™ (Cat. #R001A/B/C)
TaKaRa Taq™ Hot Start Version (Cat. #R007A/B)
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
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 area