Cat. # CY501

For Research Use

TaKaRa

CycleavePCR™ Core Kit

Product Manual

v202204Da

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I. Description

CycleavePCR Core Kit is designed exclusively for real-time PCR with Cycling Probe detection. Real-time PCR is a very useful technology which allows quick and quantitative reactions. Cycling Probe Technology allows highly specific detection. The combination of real-time PCR and Cycling Probe Technology achieves accurate and quick detection of a target gene and quantification, or detection of SNP (single nucleotide polymorphism). This kit can be used with SmartCycler System or SmartCycler II System (Cepheid) and other instruments.

II. Principle

This kit simultaneously carries out both PCR amplification with *TaKaRa Ex Taq*® HS and detection of the amplified product in real-time through Cycling Probe Technology.

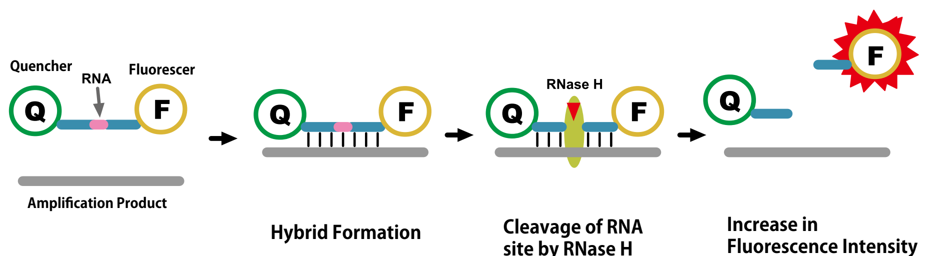
(1) PCR

PCR is a simple and powerful method to amplify a target gene *in vitro* from a tiny amount of DNA. It can amplify the target DNA fragment up to 10^6 fold in a short time by repeating the cycle composed of three incubation steps at three different temperatures; denaturation of DNA, primer annealing, synthesis of complementary chain with DNA polymerase. As this kit uses Takara Bio's PCR enzyme for Hot Start PCR, *TaKaRa Ex Taq* HS, non-specific amplification from mispriming or from primer-dimers before thermal cycling can be prevented to achieve highly sensitive detection.

(2) Cycling Probe Technology

Cycling Probe Technology is a high-sensitive detection method utilizing a combination of a chimeric probe, composed of RNA and DNA, and RNase H. The specific sequence of the target gene to be amplified can be detected efficiently during or after amplification by this method. As long as this probe remains intact, no strong fluorescence can be emitted because of the quenching function. When this probe forms a hybrid with the complementary sequence of amplified product, RNase H specifically cuts the RNA region of this probe, resulting in emission of strong fluorescence. By measuring the intensity of emitted fluorescence, the amount of amplified product can be monitored.
A probe including a mismatch in its probe region is not cut by RNase H, therefore Cycling Probe Technology allows highly specific detection and can recognize even a SNP.

Principle of Cycling Probe Technology :



III. Components (for 50 reactions)

1.	10X CycleavePCR Buffer		125 μl
2.	dNTP Mixture	2.5 mM each	150 μl
3.	Mg solution	25 mM	250 μl
4.	*TaKaRa Ex Taq* HS	5 U/μl	12.5 μl
5.	Tli RNaseH II	200 U/μl	25 μl
6.	Positive Control	10^4 copies/μl*1	10 μl
7.	Positive Control primer mix	10 μM each	10 μl
8.	Positive Control probe (FAM)*2	25X	10 μl
9.	dH_2O		700 μl

*1 Positive Control is a plasmid. Its copy number is calculated based on the OD_{260} value and it does not necessarily reflect the real molecule number.
*2 Store the fluorescent labeled probe protected from light.

Materials Required but not Provided

- Real-time PCR instrument
  - SmartCycler System (Cepheid)
  - SmartCycler II System (Cepheid)
- SmartCycler reaction tube (Cepheid)
- PCR primer*3
- Cycling probe*3
- 1,000 μl, 200 μl, 20 μl, and 10 μl micropipettes
- Micropipette tips (sterilized)

*3 Please refer to “IX. Appendix; Guideline for Designing Primers” for the method to design PCR primers.

IV. Storage

-20℃

V. Features

1. The combination of real-time PCR and cycling probe technology allows quick and accurate results of PCR reaction. This combination can be used to establish a detection system for SNP analysis which discriminates single nucleotide differences.

2. This kit uses Takara Bio's Hot Start PCR enzyme, *TaKaRa Ex Taq* HS. As this enzyme is optimized for use in real-time PCR, this product offers high amplification efficiency and allows highly sensitive detection.

VI. Precautions for Use

Please read through the following precautions prior to starting the protocol.

1. It is recommended to prepare a Master Mix (mixture of dH_2O, buffer, enzymes, etc.) for several reactions. The preparation of Master Mix minimizes the loss by pipetting, dispensing the reagents, and mixing frequency allowing accurate dispensing of reagents per reaction. Accordingly, the variance in experiments can be minimized.
2. The enzymes such as *TaKaRa Ex Taq* HS and Tli RNaseH II should be mixed gently, without generating bubbles. Prior to pipetting, please centrifuge them gently to collect reagents on the tube wall to at the bottom. As the enzymes are highly viscous containing 50% glycerol, pipetting should be carried out carefully.
3. The enzymes should be stored at -20℃ before use, and should be returned to -20℃ immediately after use.
4. For dispensing reagents, new disposable tips should be used to minimize contamination among samples.

VII. Protocol

1. Prepare the PCR reaction mixture on ice.
   Prepare the following reaction mixture in the volume of required reaction number and an additional few. Then dispense 24 μl in real-time PCR tubes.

< One reaction >

Reagent	Volume	Final Conc.
10X CycleavePCR Buffer	2.5 μl	1X
dNTP Mixture (2.5 mM each)	3 μl	0.3 mM
Mg solution (25 mM)	5 μl	5 mM
PCR Forward Primer (20 μM)	0.25 μl	5 pmol
PCR Reverse Primer (20 μM)	0.25 μl	5 pmol
Cycling Probe (5 μM)*1	1 μl	
Tli RNase H II (200 U/μl)	0.5 μl	100 U
TaKaRa Ex Taq HS (5 U/μl)	0.25 μl	1.25 U
dH_2O	11.25 μl	
Total	24 μl	

*1 Cycling Probe is generally used at 5 pmol per reaction, but the amount should be adjusted depending on the signal intensity. When used with SmartCycler, it should be adjusted to have the fluorescence intensity of 300 - 500.

2. Add 1 μl of template into the tubes prepared at step 1.
   The template can be added in a volume greater than 1 μl. In this case, the volume of dH_2O should be adjusted depending on the template volume.

3. Start the reaction.
   Gently centrifuge the tubes. Load the tubes in a real-time PCR instrument and start the reaction. Refer to the following table for setting the reaction parameters.

Step	Temp.	Time	Detection	Remark
Initial denaturation	95℃	10 - 30 sec	OFF	When the template is genomic DNA, heat denaturation for 30 sec, or possibly 1 min depending on the case, is necessary. (The reaction may not be stable with denaturation over 1 min.) Templates less than 500 bp might not require the initial denaturation.
Denaturation	95℃	5 sec	OFF	As the amplified size of the target in real-time PCR is generally less than 500 bp, 95℃ 3 - 10 sec is sufficient.
Annealing	55℃	10 - 20 sec	OFF	When non-specific product is generated or amplification efficiency is low, optimization of annealing temperature can improve. Longer annealing time can sometimes improve the amplification efficiency.
Extension	72℃	10 - 15 sec	ON	When the amplified size is around 100 bp, 10 - 15 sec is adequate. When the size is longer than 100 bp, the time can be extended by 5 sec per 100 bp.
Cycle number	30 - 50 cycles			SmartCycler terminates the reaction at the point when the amplified product is detected. This function can be utilized for quicker analysis.*2

*2 This function is available in SmartCycler Software Version 2.0. Although it is not present in SmartCycler Software Version 1.2, it can be available by upgrading with SmartCycler Software Version 2.0 Upgrade Kit. This operation does not require the upgrade of the instrument itself.

4. After the reaction terminates, perform analysis.
   After reaction completion, confirm the amplification curve and perform analysis.*3

*3 Please refer to the instruction manual of the real-time PCR instrument, and "VIII. Experimental Example" for the analysis method in SmartCycler.

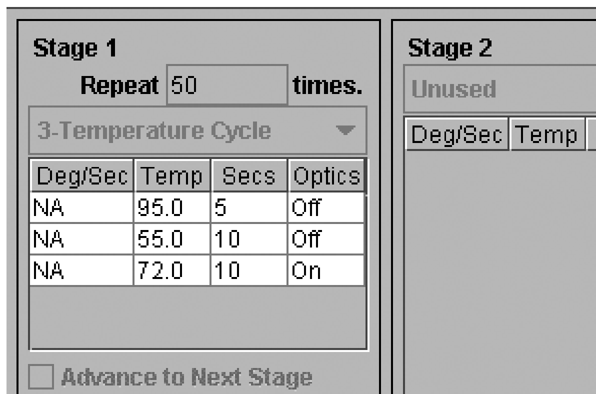
VIII. Experimental Example

CycleavePCR reaction using Positive Control (SmartCycler II)
The supplied Positive Control allows verification that the operation proceeded correctly.

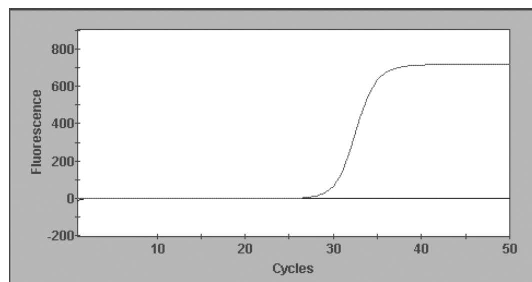
1. PCR reaction
   Prepare the reaction mixture including Positive Control Primer mix and Positive Control probe in each 1 μl, by following the protocol described in "VII. Protocol". The composition of reaction mixture is as below.

Reagent	Volume
10X CycleavePCR Buffer	2.5 μl
dNTP Mixture (2.5 mM each)	3 μl
Mg solution (25 mM)	5 μl
Positive Control primer mix	1 μl
Positive Control probe	1 μl
Tli RNase H II (200 U/μl)	0.5 μl
TaKaRa Ex Taq HS (5 U/μl)	0.25 μl
Positive Control	1 μl
dH_2O	10.75 μl
Total	25 μl

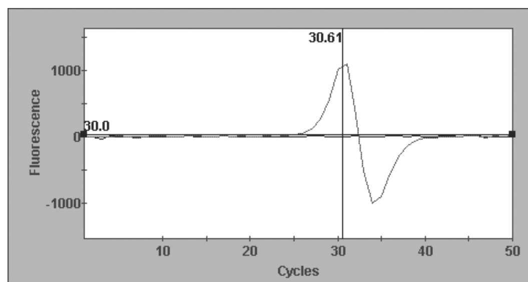
2. Set the reaction parameter as shown below.



3. Example of Positive Control
   The following result is expected in case of a reaction with Positive Control.



Amplification curve



2nd derivative

IX. Appendix

Guideline for Designing Primers

Designing primers specific to a reaction is essential to achieve highly sensitive detection via real-time PCR. Please refer to the following guidelines for primer design.

Amplified Product

Amplified size	100 - 150 bp is recommended.	Fragments of up to 300 bp can be amplified efficiently.
Tm	< 90℃	Higher temperature may lower the reaction efficiency.

Primer

Length	17 - 25 mer	
GC content	40 - 60 %	
Tm	55 - 65℃	
	Tm values of Forward primer and Reverse primer must not differ largely.	
Sequence	3' termini should not have three serial G or C.	It can lead to low specificity in annealing, thus non-specific products would be formed, e.g. primer dimers.
	3' termini should not be T.	It can lead to mismatch annealing. The specificity would be low.
Complementarity	The internal sequence of the primer should not have more than 2 bases the same as that of 3'-termini.	It can form primer dimers.
Specificity	Perform BLAST search to confirm the primer specificity.	https://blast.ncbi.nlm.nih.gov/Blast.cgi

X. Reference

F. Bekkaoui, *et al*. *BioTechniques*. (1996) **20**: 240-248.

XI. Related Products

CycleavePCR™ Reaction Mix (Cat. #CY505A/B)*
Probe qPCR Mix (Cat. #RR391A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)

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

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