Cat. # RR385S
RR385A

For Research Use

TaKaRa

*Bca*BEST® Isothermal FluorDetect Kit (DNA/RNA)

Product Manual

v202504Da

Table of Contents

I. Description

The *Bca*BEST Isothermal FlourDetect Kit (DNA/RNA) provides a rapid solution for fluorescent detection of a target nucleic acid sequence from DNA or RNA via loop-mediated isothermal amplification (LAMP). LAMP is type of isothermal amplification that employs a DNA polymerase with strand-displacing activity and four to six primers to amplify a target nucleic acid sequence from minute amounts of template DNA. One-step RT-LAMP can also be performed with a mix of a strand-displacing DNA polymerase and a reverse transcriptase when the template is RNA.

Two components are provided with the *Bca*BEST Isothermal FlourDetection Kit (DNA/RNA): *Bca*BEST Isothermal Mix (2X) and TB Green® Solution (300X). *Bca*BEST isothermal Mix (2X), which contains premixed *Bca*BEST DNA Polymerase ver.2.0 and PrimeScript™ III Reverse Transcriptase, allows for amplification of both DNA and RNA templates. TB Green Solution (300X) is an intercalating dye that enables detection of amplified product by fluorescence with a real-time PCR system (using a FAM filter).

To amplify a target sequence, DNA or RNA template, primers, *Bca*BEST Isothermal Mix (2X), and TB Green Solution are mixed. The reaction is then incubated under isothermal conditions at 63℃ for 20 min using a real-time PCR system. *Bca*BEST Isothermal Mix (2X) also contains Uracil-*N*-Glycosylase (UNG) to prevent false positives due to carryover contamination.

II. Components [20 reactions (RR385S)/100 reactions (RR385A), 25 μl volume per reaction]

	Cat. #RR385S	RR385A
*Bca*BEST Isothermal Mix (2X)*	250 μl	625 μl x 2
TB Green Solution (300X)	50 μl	50 μl

* Contains pyrophosphatase (inorganic), which degrades the pyrophosphate produced in the amplification reaction, so amplification cannot be detected by turbidity.

III. Storage

-20℃

IV. Materials Required but not Provided

1. Real-time PCR system
2. Dedicated reaction tubes or plates
3. LAMP primers*1,2
4. RNase-free water
5. Micropipettes and tips

*1 The TB Green intercataling dye enables detection of amplfied products via flourescence detection using a FAM filter.

*2 Common LAMP primers can be used. Although LAMP can be performed using four basic primers (F3/B3/FIP/BIP), using the optional primers (LoopF/LoopB) can greatly improve reaction efficiency.

V. Precautions for Use

Read these precautions before use and follow them when using this product.

1. Thaw *Bca*BEST Isothermal Mix (2X) on ice, mix gently, and spin down before use. After use, immediately store at -20℃.
2. Wear gloves when handling TB Green Solution, as it binds to nucleic acids and thus should be handled as a possible mutagen.
3. When dispensing reagents, be sure to prevent inter-sample contamination by using a new disposable tip.
4. We recommend preparing a small amount of extra reaction mixture (consisting of *Bca*BEST Isothermal Mix (2X), 10X LAMP Primer Mix, 25X TB Green Solution, and RNase-free water) to minimize variability within your experiments.
5. Do not open, close, or autoclave tubes after amplification reactions have completed. This can lead to nucleic acid contamination within your laboratory.

VI. Protocol

Optimal primer concentration, reaction time, reaction temperature, and TB Green Solution concentration vary depending on the target gene and primer sequences. We recommend starting with the following standard protocol and adjusting experimental conditions as needed.

[Standard Protocol]

1. Prepare 10X LAMP Primer Mix and 25X TB Green Solution.

   < 10X LAMP Primer Mix (2.5 μl per reaction) >
   FIP/BIP primers : 16 μM
   F3/B3 primers : 2 μM
   LoopF/B primers : 8 μM

   < 25X TB Green Solution (prepared at time of use) (1 μl per reaction) >

Reagent	Volume
RNase-free water	27.5 μl
TB Green Solution (300X)	2.5 μl
Total	30.0 μl

2. Prepare the following reaction mixture on ice.

   < Per reaction >

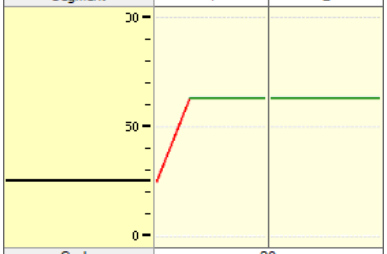
Reagent	Volume	Final conc.
*Bca*BEST Isothermal Mix (2X)	12.5 μl	1X
10X LAMP Primer Mix	2.5 μl	1X
25X TB Green Solution	1.0 μl	1X
Template (DNA/RNA)*	x μl	
RNase-free water	up to 25 μl	

   * To prevent non-specific amplification during preparation of the reaction mixture, add the template last.

3. Perform the LAMP/RT-LAMP reaction.
   1) Spin down the reaction tube or plate gently.
   2) Incubate for 20 min at 63℃.
   3) Use fluorescence detection with a FAM filter to confirm amplification.

   Note: If carryover contamination is suspected, incubate the reaction mixture for 10 min at 25℃ prior to the reaction. This step will activate the UNG within the reaction mixture to degrade carryover amplification products. This step is typically not required when contamination is not suspected.

< Program settings when performing detection using a real-time PCR system >

Pattern	2 Step PCR	
Segment	1	2
		
Cycle	20	
Temperature(deg)	63.0	63.0
Hold Time(mm:ss)	00:30	00:30
Data Collection	☐	☑
Ramp Rate(deg/sec)	Default	Default
Increment Temp(deg)	0.0	0.0
Increment Time(sec)	0.0	0.0

Example program settings

Two-Step PCR

Cycles: 20

63℃ 30 sec

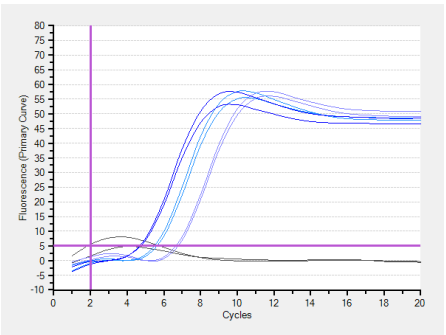
63℃ 30 sec (data acquisition: FAM filter)

The above settings allow fluorescence data to be collected every minute over the 20-min amplification reaction. A melting curve analysis step after the isothermal reaction can be added as necessary.

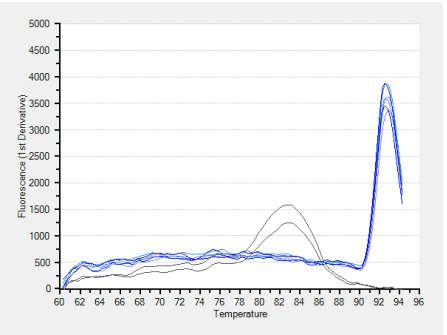
< Example amplification and detection >

Note: A Thermal Cycler Dice™ Real Time System III (Cat. #TP950) was used to generate the data below.

Amplification curve



Melting curve



If the amplification curve rises too quickly and the fluorescence value after amplification is displayed as zero, use the manual settings to adjust the baseline. Setting the baseline and threshold appropriately allows detection time to be output as a Ct value.

Furthermore, the melting curve can be used to check the degree of uniformity of the amplified products. Nucleic acid Tm value is affected by chain length and GC content, among other factors, so different amplification products can be identified in many cases.

VII. Troubleshooting

[Amplification is observed in negative control samples]

Problem	Possible causes	Solutions
Amplification curve rises slower than in positive control samples Melting curve Tm value and shape are different from those of positive control samples	Nonspecific amplification	• Shorten reaction time • Consider increasing the reaction temperature by 1 - 5℃. • Consider increasing or decreasing the primer concentration • Consider changing the primer set • Prepare reaction mixture on ice
Amplification is observed at about the same time as in positive control samples Melting curve Tm value and shape are the same as those of positive control samples	Carryover contamination	• Add a UNG degradation step before the reaction • Keep separate work areas for reagent preparation and running the reaction • Clean work areas and apparatus

Caution: LAMP is highly reactive, so nonspecific amplification may be detected even in negative control reactions.

Be particularly careful to prevent carryover contamination of previously amplified products.

[No amplification is observed in positive control samples, poor amplification efficiency]

Problem	Possible causes	Solutions
No amplification is observed	Fluorescence not detected Reaction is not occuring	• Verify that fluorescence data is being acquired • Consider changing the primer set • Re-purify DNA/RNA template
Poor amplification efficiency	Reaction conditions are not optimal	• Add loop primers if not already used • Consider increasing or decreasing the primer concentration. • Check the reaction temperature • Consider changing the primer set • Reduce TB Green Solution concentration

VIII. References

1) Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. Loop-mediated isothermal amplification of DNA. *Nucleic acids research*. (2000) **28**(12): e63.
2) Nagamine, K., Hase, T., & Notomi, T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and cellular probes*. (2002) **16**(3): 223-229.

IX. Related products

*Bca*BEST® DNA Polymerase ver.2.0 (Cat. #RR380A/B)
TB Green® Solution (Cat. #9300A)
RNase-free Water (Cat. #9012)

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 from our website at www.takarabio.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.