

Real-time PCR reagents make long-awaited debut! Primer-dimer production suppressed to the limit!

SYBR® Premix DimerEraser™ (Perfect Real Time) (RR091)

- **Excellent specificity:** Result of a search for maximum specificity.
- **Convenient:** Reagents of Perfect Real Time series are pre-mixed.

Real-time PCR with SYBR® Green I detection is a low-cost and simple experimental method, but production of primer-dimers may disturb accurate quantification. SYBR® Premix DimerEraser™ (Perfect Real Time) has just joined Perfect Real Time series including highly efficient SYBR® Premix Ex Taq™ (Perfect Real Time) (RR041) and highly specific SYBR® Premix Ex Taq™ II (Perfect Real Time) (RR081). The new product is the result of a search for maximum specificity. The product includes TaKaRa Ex Taq® HS, an enzyme for Hot Start PCR, which prevents mispriming before PCR cycles, as well as Takara's unique accessory protein and a modified buffer, which efficiently suppress mispriming during PCR cycles. In some reaction conditions where SYBR® Premix Ex Taq™ II fails complete suppression of primer-dimer formation, SYBR® Premix DimerEraser™ can more clearly succeed. As a result, trace amounts of target molecules can be detected at high-specificity and high-sensitivity, allowing reliable quantification in a wide range of template concentrations.

Experimental example

[Method]

The specificity was compared amongst SYBR® Premix DimerEraser™ (RR091), traditional SYBR® Premix Ex Taq™ II (RR081) and a competitor's product, using 2 pairs of primers prone to form primer-dimers (A and B) and another pair of those prone to produce non-specific high molecular weight by-product (C).

[Results]

(1) Suppression of primer-dimer production:
Primer pairs A and B

PCR reaction was performed according to the standard protocol of each product. As a result, SYBR® Premix Ex Taq™ II and the competitor's product produced trace amounts of non-specific amplification products including primer-dimers. On the other hand, SYBR® Premix DimerEraser™ achieved highly specific reactions, producing no non-specific amplification products (Figure 1).

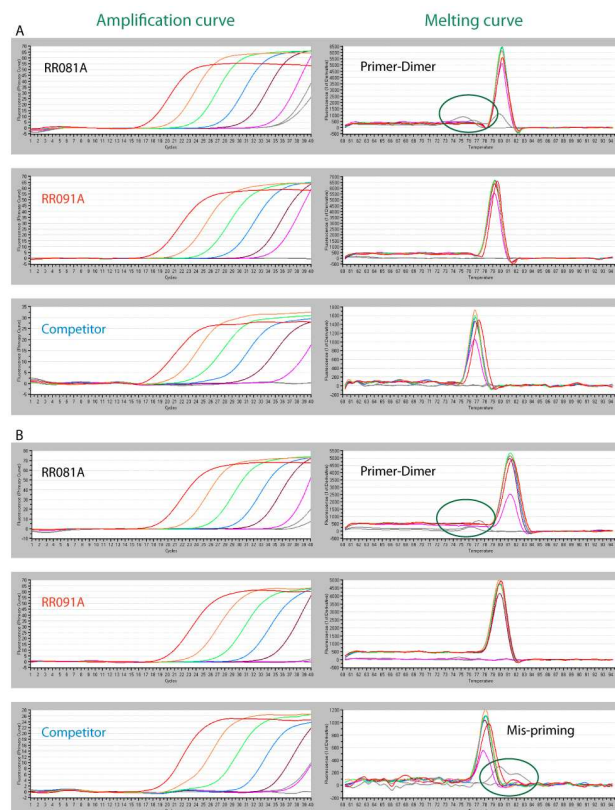


Fig 1: Comparison example of reaction specificity #1. Template : Serial dilution of cDNA (equivalent to HL60 cells or rat liver total RNA 1pg-100 ng; 1 reaction each) or H₂O (NTC, in duplicate). Target: (A) Human YWHAZ. (B) Rat Atp5f1. Instrument: Thermal Cycler Dice™ Real Time System (not available in Europe). Reaction conditions: As recommended by the manufacturer.

[RR081A] 95°C 30 sec. > 40 cycles (95°C 5 sec. / 60°C 30 sec.) > denaturation analysis
 [RR091A] 95°C 30 sec. > 40 cycles (95°C 5 sec. / 55°C 30 sec. / 72°C 30 sec.) > denaturation analysis
 [competitor] 95°C 15 min. > 40 cycles (94°C 15 sec. / 55°C 30 sec. / 72°C 30 sec.) > denaturation analysis

(2) Suppression of non-specific template-origin amplification: Primer C

Non-specific high molecular weight by-products are occasionally produced depending on the sequence for PCR amplification or amount of a template (usually, a peak is observed at a T_m value higher than the target one). The standard protocol of SYBR® Premix DimerEraser™ consists of three PCR steps, but in such cases, the optional protocol consisting of two PCR-steps is effective. In this experimental example, SYBR® Premix DimerEraser™ achieved complete suppression of non-specific amplifications under the optional protocol (Figure 2).

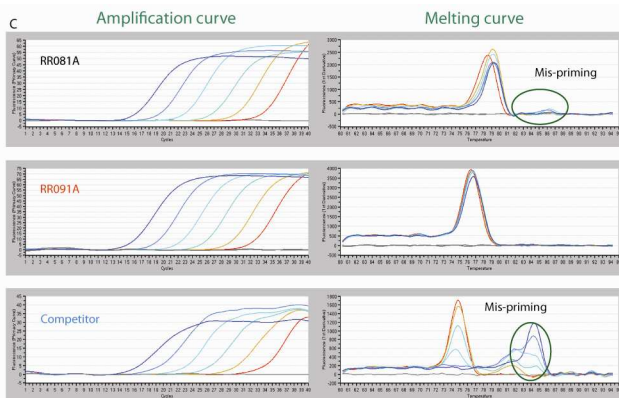


Fig 2 : Comparison example of reaction specificity #2. Template : Serial dilution of cDNA (equivalent to HL60 cells total RNA 1 pg-100 ng; 1 reaction each) or H₂O (NTC, in duplicate) Target: (C) Human GAPDH. Instrument: Thermal Cycler Dice™ Real Time System (not available in Europe). Reaction conditions: As recommended by the manufacturer.

[RR081A] 95°C 30 sec. > 40 cycles (95°C 5 sec. / 60°C 30 sec.) > denaturation analysis
 [RR091A] (optional 2-step protocol) 95°C 30 sec. > 40 cycles (95°C 5 sec. / 60°C 30 sec.) > denaturation analysis
 [competitor] 95°C 15 min. > 40 cycles (94°C 15 sec. / 55°C 30 sec. / 72°C 30 sec.) > denaturation analysis

Takara Bio is now offering three types of real-time PCR reagents for SYBR® Green I detection, including SYBR® Premix DimerEraser™ (Perfect Real Time) presented above, each of which has different features in terms of specificity and amplification efficiency.

Experimental example

[Method]

Real-time PCR was performed with the three types of SYBR® Premix, using λDNA as a template, and then specificity and amplification efficiency were compared among the three types of Premix reagents.

Although the reaction was carried out under the recommended conditions of each reagent, the target size for PCR amplification in the reaction was 300 bp, which was longer than the recommended size (80-150 bp), and thus the conditions were a little difficult for amplification.

[Results/Discussion]

- Specificity

SYBR® Premix Ex Taq™ produced a non-specific amplification product (which was considered to be primer-dimer) in one of two No-Template-Control (NTC) reactions, while SYBR® Premix Ex Taq™ II and SYBR® Premix DimerEraser™ did not produce such a product in the same reactions (Figure 3).

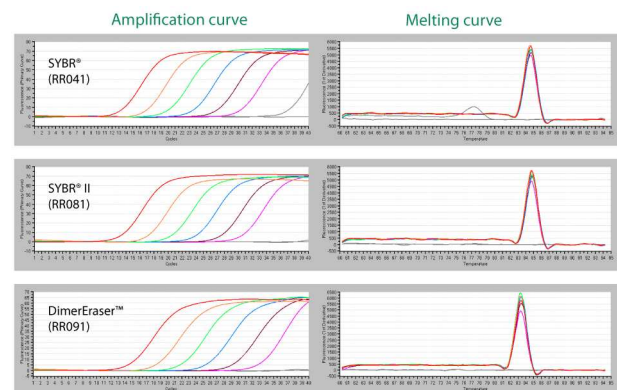


Fig 3 : Comparison experiment using the various Premixes from the SYBR® Premix Reagent Trial Pack. Reagents: 3 types of SYBR® Premix. Template: λ DNA 1 fg, 10 fg, 100 fg, 1 pg, 10 pg, 100 pg (1 reaction each) NTC (duplicate reactions). Target length: 300 bp. Instrument: Thermal Cycler Dice™ Real Time System (not available in Europe). PCR conditions: as recommended for each Premix.

- Amplification efficiency

The PCR amplification efficiency rates obtained from the calibration curve indicated that SYBR® Premix Ex Taq™ and SYBR® Premix Ex Taq™ II achieved more efficient amplification than SYBR® Premix DimerEraser™ (Figure 4).

In a comprehensive consideration of the said results, SYBR® Premix Ex Taq™ II seems to be suitable for this reaction.

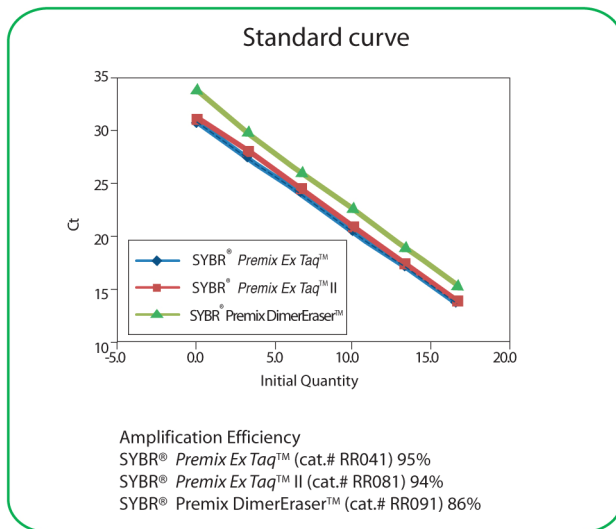


Fig 4 : Example of comparison experiment among 3 SYBR Premixes (Standard curve and amplification efficiency)

Summary (troubleshooting in real-time PCR)

As for real-time PCR experiments, many questions about designing of experimental systems have been received. Some primers can yield desired reactions with any reagent, but on the contrary, once any trouble occurs, its resolution can be difficult. Even though primers are designed according to the same parameter setting, the actual reaction results may vary among them. It seems that many investigators have encountered such troubles and had difficulties optimizing the experimental conditions.

Many real-time PCR reagents are formulated as 2-fold concentrated pre-mixed solutions to simplify experimental procedures. Such formulations may be handy, but at the same time have inconvenient aspects in optimization of the experimental conditions, for instance, magnesium concentration cannot be changed manually. SYBR[®] Premix series presented here have been developed to solve the dilemma so that reagents suitable for individual experimental systems can be found easily.

If a researcher wishes to take advantage of SYBR[®] Premix series, it is recommended to select the base reagent first. Then, if any trouble occurs in an individual experimental system, it is recommended to switch the reagent to the other one corresponding to the trouble. Please refer to the following suggestions, which may help you design a real-time PCR experimental system best suit you.

1. Selection of the base reagent

First, please try SYBR[®] Premix Ex Taq[™] (RR041) or SYBR[®] Premix Ex Taq[™] II (RR081), either of which can realize quick reaction, according to the 2-step standard protocol. A guide for selection of the most appropriate product is shown in Figure 5.

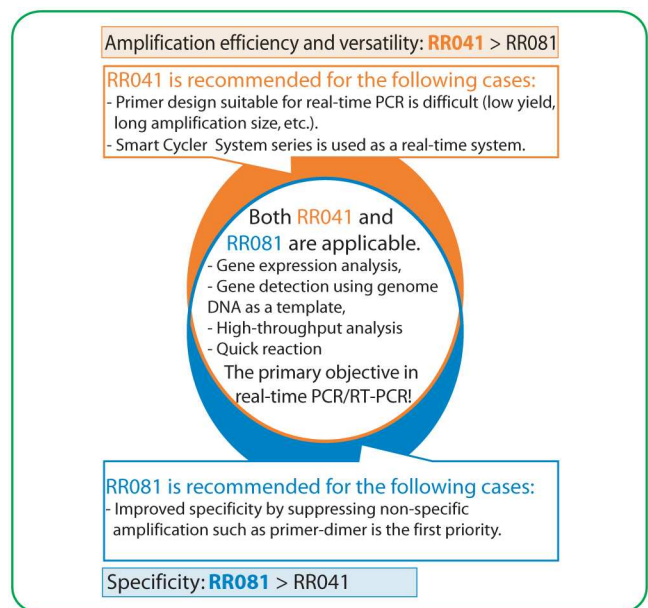


Fig 5 : Guideline for selection of RR041 or RR081

Amplification efficiency and versatility:
RR041 > RR081

RR041 is recommended for the following cases

- Primer design suitable for real-time PCR is difficult (low yield, long amplification size, etc.).
- Smart Cycler[®] System series is used as a real-time PCR system.

Both RR041 and RR081 are applicable.

- Gene expression analysis
 - Gene detection using genome DNA as a template
 - High-throughput analysis or,
 - Quick reaction
- is the primary objective in real-time PCR/RT-PCR.

RR081 is recommended for the following cases

- Improved specificity by suppressing non-specific amplification such as primer-dimer is the first priority.

If non-specific amplification causes a trouble even with RR081, please try SYBR® Premix DimerEraser™ (RR091) according to the standard protocol (3-step PCR). Furthermore, if non-specific high molecular weight amplification product is observed, please try RR091 according to the option protocol (2-step PCR)*.

* Amplification efficiency under the option protocol of RR091 may be low compared with the standard protocol. Thus, it is recommended to examine the reaction according to the standard protocol first.

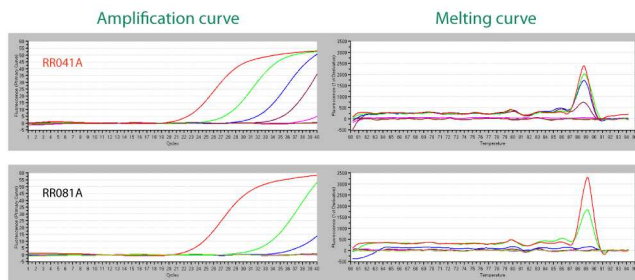


Fig 6 : Example of difficult target amplification.

2. Features of respective products

A wise choice of SYBR® Premix series leads to a quick resolution of any trouble.

- SYBR® *Premix Ex Taq*™

There are successful cases of outstandingly efficient amplification of difficult sequences,

which had failed to be amplified with competitor's real-time PCR reagents.

An article (Shaik, GM., et al., [2008] *Nucleic Acids Res.*,36[15], e93.) reported that mouse Thy-1 sequence (864 bp) had failed to be amplified with commercial reagents. Takara Bio tried amplification of the concerned sequence.

PCR with SYBR® *Premix Ex Taq*™ under conditions of the article resulted in successful amplification (Figure 6, top). The bottom figures show results of PCR with SYBR® *Premix Ex Taq*™ II under the same conditions.

- SYBR® *Premix Ex Taq*™ II

SYBR® *Premix Ex Taq*™ II is a handy reagent, because of its good balance of amplification efficiency and specificity.

- SYBR® *Premix DimerEraser*™

SYBR® *Premix DimerEraser*™ is a result of pursuit for the maximum specificity. The reagent will be an ultimate resolution for any trouble of non-specific amplification such as primer-dimer.

The product is designed for 3-step PCR as the standard protocol, and thus needs more time compared with SYBR® *Premix Ex Taq*™ and SYBR® *Premix Ex Taq*™ II, but shows excellent specificity instead.

Ordering Information

Cat. No.	Product name	Size
RR041A	SYBR® <i>Premix Ex Taq</i> ™ (Perfect Real-Time)	200 x 50µl reactions
RR081A	SYBR® <i>Premix Ex Taq</i> ™ II (Perfect Real-Time)	200 x 50µl reactions
RR091A	SYBR® <i>Premix DimerEraser</i> ™ (Perfect Real-Time)	200 x 50µl reactions

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