

FAQs

SYBR Premix Ex Taq™

1) I'm new at real time PCR, and I'm trying to estimate how many reactions I will need to perform when running my qPCR reactions. I have two different cell lines, and want to characterize 3 different genes in each. What number of reactions can I expect to run?

One package of SYBR Premix Ex Taq (Perfect Real Time) is sufficient reagent for 200 reactions (50 µl size).

A standard curve is required for each gene. Generally, for one gene, at least 7 standards (6 data points plus a negative control) in addition to the sample should be performed.

In this case, for each of the 3 genes, a standard (composed of 7 data points) plus 2 experiment samples for each cell line = $3 \times (7 + 2) = 27$ reactions would be performed. Therefore, at least 27 reactions will be required.

2) How many reactions (points) are recommended for a typical standard curve?

Typically, at least 6 reactions (6 points) are used to establish the standard curve, plus dH₂O for a negative control. Takara has used cDNAs which corresponded to 1 pg, 10 pg, 100 pg, 1 ng, 10 ng and 100 ng of mouse liver total RNA respectively (and dH₂O for negative control).

If possible, establish the standard curve within a Ct range of ~ 15-35.

3) What PCR product size is optimal for real-time?

A size range of 80-150 bp is the most recommended amplification size for real time PCR, although sizes up to 300 bp are possible. Please refer to the "Guideline for Designing of Primers" on page 13 in our SYBR Premix Ex Taq manual.

Note, however, that one of our customers has reported successful quantitation of an amplified 800 bp fragment with this kit using an ABI PRISM. However, Takara still recommends performing 80-150 bp amplifications for best quantification.

4. What recommendations do you have for using SYBR Premix Ex Taq with an MJ Research DNA Engine Opticon ?

Typically, 10 µl or 20 µl reactions are performed using our standard cycling conditions (refer to the protocol for the LightCycler on page 9 of our product manual for information regarding the composition of reaction mixture and the cycling conditions).

Set your final concentration of primers at 0.2 µM for your first trial.

Perform the initial denaturation at 95°C for 10 seconds. DO NOT perform a longer initial denaturation.

Finally, first try using the shuttle PCR method [95°C/5 sec, 60°C/20 seconds; 35-45 cycles] before trying the 3-step PCR method if your primers and target size agree with our "Guideline for Designing of Primers" on page 13 of the product manual.

5. Can SYBR® Premix Ex Taq™ be mixed with an RT-PCR enzyme so that real time analysis of cDNA can be performed in one step? Is SYBR® Premix Ex Taq™ compatible with an RT polymerase?

We do not recommend using SYBR® Premix Ex Taq™ for real time one-step RT-PCR by using it in conjunction with an RTase. Using it in this fashion will not provide the expected performance of this product.

Instead, we recommend using Takara's Real Time One Step RNA PCR Kit, Version 2.0 (TAK RR026A) for real time analysis of cDNA in one step.

6. I have noticed a green precipitate at the bottom of my SYBR® Premix Ex Taq™ tube. I'm assuming that the SYBR® Green I dye has precipitated out of solution. Is there a good way to get the SYBR Green I back into solution?

A greenish-yellow precipitate sometimes can be observed in our SYBR® Premix Ex Taq™ when stored at -20°C. When this occurs, dissolve the precipitate completely by mixing the Premix gently after letting the tube stand at room temperature for several minutes, protected from light, or by warming with your hands. Do not vortex!!

We have verified that this product shows good performance after the precipitate is dissolved completely.

7. What is the magnesium concentration of SYBR® Premix Ex Taq™?

Unfortunately, the Mg²⁺ concentration of this product is proprietary.

8. What is the ROX that is included with the SYBR® Premix Ex Taq™? For what purpose is it used?

ROX (i.e. Carboxy-X-Rhodamine) is a convenient internal reference standard for use in normalizing signals due to non-PCR related fluorescence fluctuations that occur either between wells or over time.

Beginning in 2005 please note that two types of ROX Reference Dye (Original Version ROX and ROX II) are supplied with this product. For normalization when using ABI PRISM 7000 series instruments, please use the Original Version ROX.