

RAPID CRS Evidence Sheet – Real-time PCR–

【Abstract】

Few plate seal for PCR has DNA free and DNase/RNase certificate. Therefore, we developed a certified seal, RAPID CRS. The compatibility of the RAPID CRS with qRT-PCR (SYBR Green Method) was evaluated through comparison of qRT-PCR reactions between two sealing films, RAPID CRS and a seal recommended by a PCR instrument manufacturer. The same result between them showed that the RAPID CRS is useful for qRT-PCR.

【Methods】

Firstly, we generated cDNA from mRNA extracted human normal cells and ran qRT-PCR reactions with each pair of two genes primers. Secondly, we used KAPA SYBR FAST qPCR Kit (Kapa Biosystems, Inc.) and pipetted samples into a 96-well plate. Thirdly, we sealed the right-half side of the plate with RAPID CRS and the left-half side of plate with a seal recommended by a PCR instrument manufacturer. Finally, we ran qRT-PCR by using TaKaRa PCR Thermal Cycler Dice and compared results between them in triplicate.

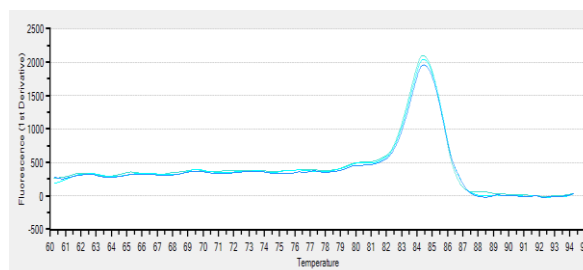
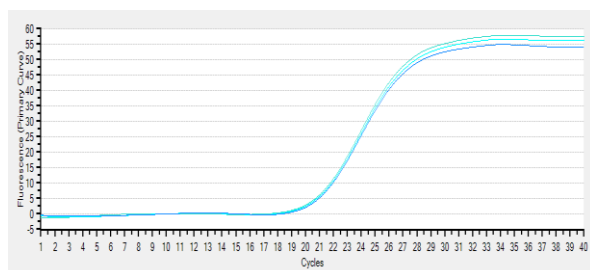
【Results】

The results showed similar PCR reactions for gene 1 and 2 despite different sealing films (Figure 1, Table 1). Little difference of cycle threshold values in triplicate showed that RAPID CRS is as good as the recommended sealing film.

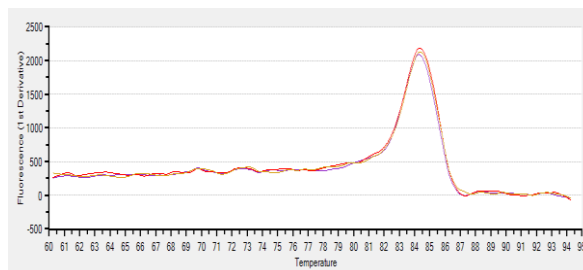
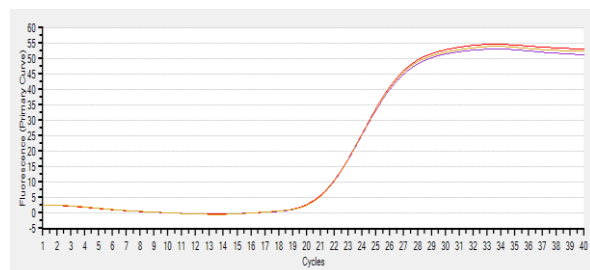
<Figure 1> Amplification Plots & Dissociation Curve

Gene1

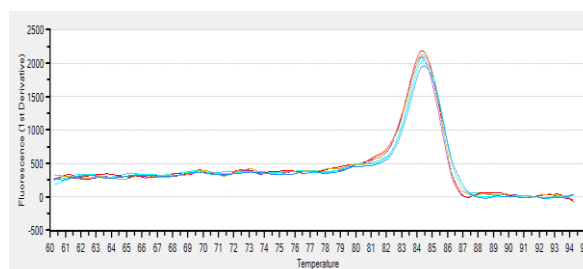
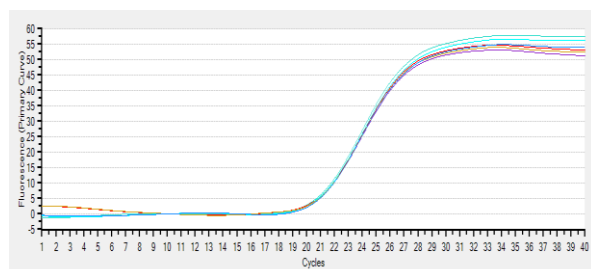
RAPID CRS



Manufacturer's Recommended Sealing Film

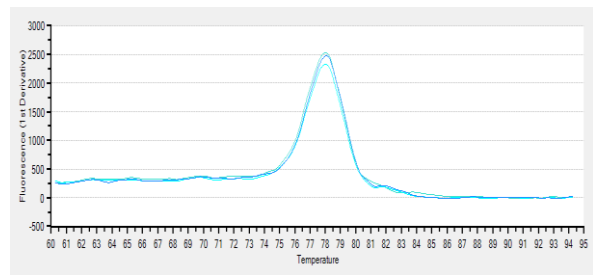
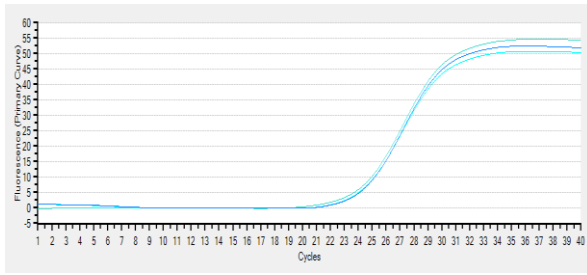


Comparison of merged data of two results above

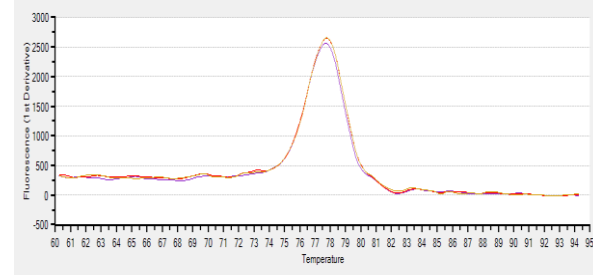
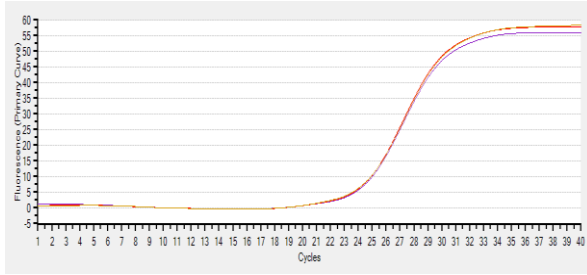


Gene2

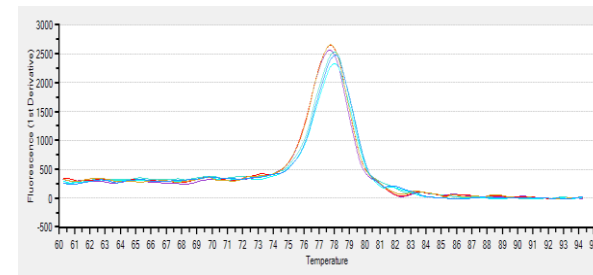
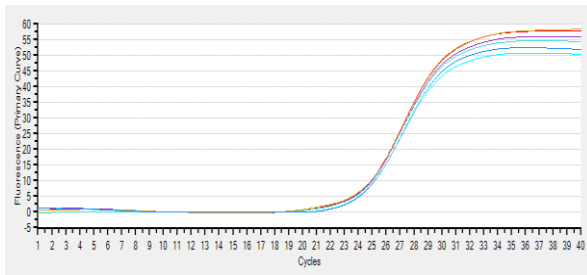
RAPID CRS



Manufacturer's Recommended Sealing Film



Comparison of merged data of two results above



<Table 1> Cycle Threshold values (2nd Derivative maximum method)

Gene1

| Samples | Ct (SDM) | Average |
|--------------------|----------|---------|
| RAPID CRS 1 | 21.29 | 21.30 |
| RAPID CRS 2 | 21.35 | |
| RAPID CRS 3 | 21.25 | |
| Recommended Seal 1 | 21.32 | 21.30 |
| Recommended Seal 2 | 21.32 | |
| Recommended Seal 3 | 21.26 | |

Gene2

| Samples | Ct (SDM) | Average |
|--------------------|----------|---------|
| RAPID CRS 1 | 24.81 | 24.87 |
| RAPID CRS 2 | 24.75 | |
| RAPID CRS 3 | 24.78 | |
| Recommended Seal 1 | 24.82 | 24.78 |
| Recommended Seal 2 | 24.86 | |
| Recommended Seal 3 | 24.92 | |

SYBR is a registered Trademark of Molecular Probes, Inc.



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