NIPPON Genetics EUROPE

Evaluation by real-time PCR:

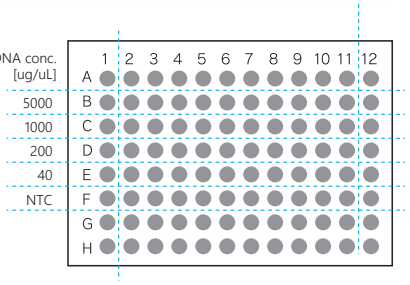
FastGene® PCR adhesive clear seal (FG-93AC) and FastGene® qPCR pressure clear seal (FG-95PC).

Purpose: Test whether FastGene® PCR adhesive clear seal is suitable for real-time PCR; Test was carried out in comparison with the commercially available competitor Fs qPCR pressure clear seal.
Evaluated products: FastGene® PCR adhesive clear seal (FG-93AC) and FastGene® qPCR pressure clear seal (FG-95PC)

Cat. No. FG-93AC; FG-95PC

PCR conditions

qPCR instrument:	StepOnePlus Life Technologies (ABI)
Plate:	Competitor Fs 96-well plate
Evaluated Seals:	1) FastGene® PCR adhesive clear seal (FG-93AC)
	2) FastGene® qPCR pressure clear seal (FG-95PC)
Comparison product:	3) Competitor Fs qPCR pressure clear seals
qPCR reagent:	KAPA SYBR Fast qPCR Kit
Template DNA:	Roche Human Genomic DNA (# 11691112001) 5.0 ng / µl
Primer:	Act-F1, Act-R1 (10 µM) < ß-actin: 294 bp amplicon
	Act-F1: TCACCCACACTGTGCCCATCTACGA
	Act-R1: CAGCGGAACCGCTCATTGCCAATGG



Reaction components

2xMaster Mix	10 µl
H_2O	7.6 µl
Primer F (10µM)	0.2 µl
Primer R (10µM)	0.2 µl
Template DNA	2.0 µl
total	20 µl

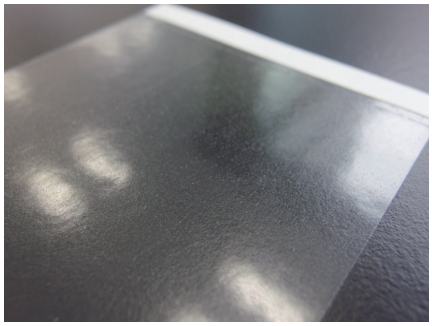
Cycle program (StepOne default fast cycle)

Initial Denaturation:	95 °C 20 sec	
Denaturation:	95 °C 3 sec	
Anneal/Extension:	60 °C 30 sec (detection)	40 cycle
Melting Curve:	95 °C 15 sec	
	60 °C 1 min	
	95 °C 15 sec (+0.3 °C step detection)	
Cycle simulation:	20 µl	

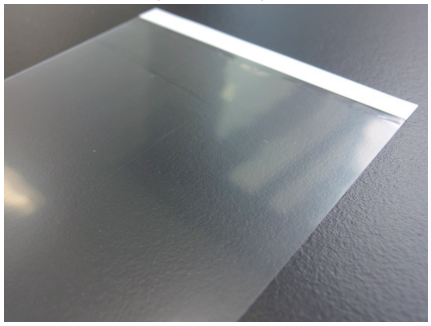
Evaluation of appearance

Evaluated products

1) FastGene® PCR adhesive clear seal (FG-93AC)

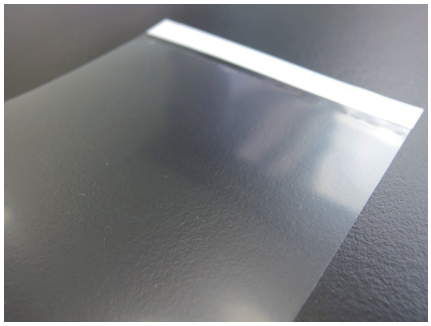


2) FastGene® qPCR pressure clear seal (FG-95PC)



Comparison product

3) Competitor Fs qPCR pressure clear seal



Compared to the both pressure seals, irregularities at the adhesive clear were observed on the surface of the seal. This irregularity is presumably caused by the adhesive material (glue).

Binsfelder Straße 77,
52351 Düren, Germany
TEL +49 2421 55 496 0
FAX +49 2421 55 496 11
Info@nippongenetics.eu
www.nippongenetics.eu

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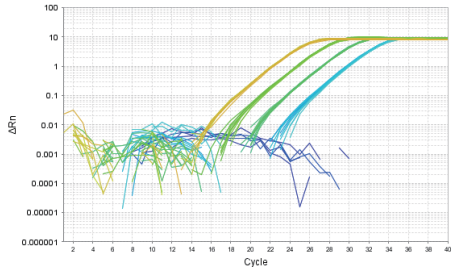
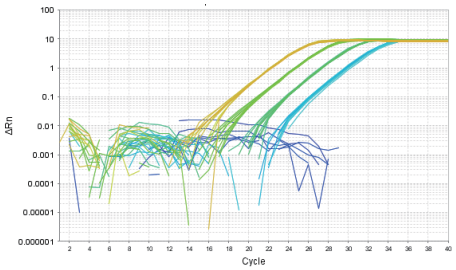
Evaluation of amplification curves

1) FastGene® PCR adhesive clear seal (FG-93AC)



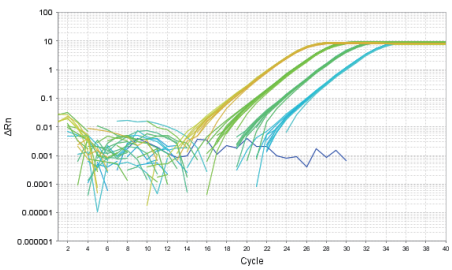
Conc. [ug/uL]	Ct	Ct SD
5000	23.3	0.09
1000	25.82	0.08
200	28.31	0.07
40	30.96	0.11

2) FastGene® pressure clear seal (FG-95PC)



Conc. [ug/uL]	Ct	Ct SD
5000	23.38	0.07
1000	25.88	0.07
200	28.46	0.09
40	31.04	0.18

3) Competitor Fs pressure clear seal

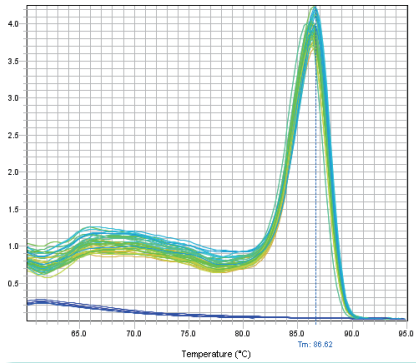


Conc. [ug/uL]	Ct	Ct SD
5000	23.3	0.09
1000	25.81	0.12
200	28.31	0.11
40	30.89	0.09

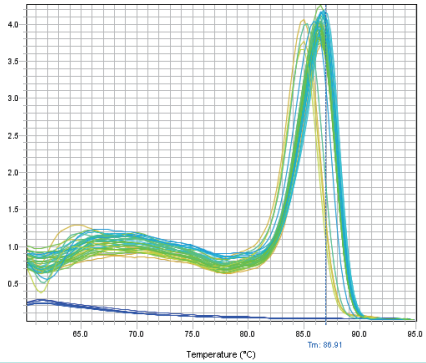
- The qPCR amplification curves are in the same range for all three seals. No significant difference could be observed between the seals. The comparison of the C_t and C_t SD show for all three seals very similar values.

Evaluation of the melting curve

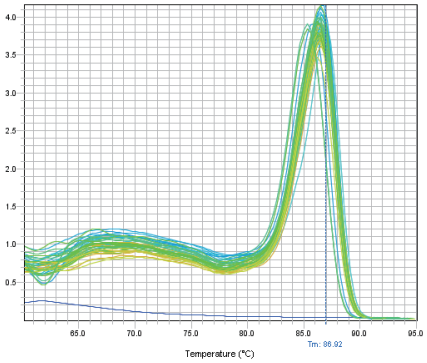
1) FastGene® PCR adhesive clear seal (FG-93AC)



2) FastGene® pressure clear seal (FG-95PC)



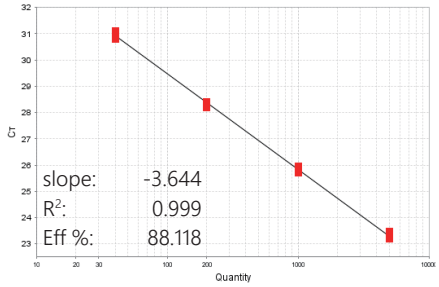
3) Competitor Fs pressure clear seal



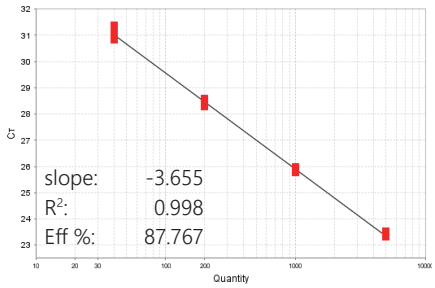
- The results for the melting curve are almost the same.

Evaluation of the calibration curve

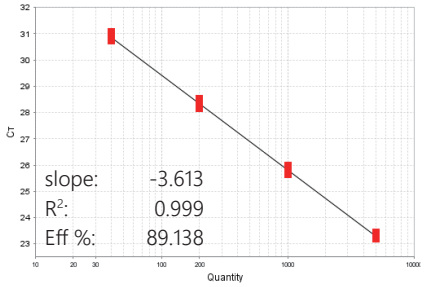
1) FastGene® PCR adhesive clear seal (FG-93AC)



2) FastGene® pressure clear seal (FG-95PC)



3) Competitor Fs pressure clear seal



- The calibration curve of the three different sheets show no difference in regards to slope, efficiency or variation

Conclusion

According to the results, FastGene® PCR adhesive clear seal (FG-93AC) showed similar performance compared to the other two qPCR pressure seals. The seal can therefore be used without restrictions in our qPCR experiments.

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu

TEL +49 2421 55 496 0 FAX +49 2421 55 496 11 Info@nippongenetics.eu