

Customer feedback on products

Product Name	• KAPA HiFi HotStart ReadyMix (KK2601)
Manufacturer	• KAPA BIOSYSTEMS
Application	• Validation of the effect of siRNA-mediated knockdown of IFNγ-induced IDO (indoleamine 2,3-dioxygenase) in primary-cultured mouse hepatocytes

The following data were provided by the courtesy of Dr. Hitoshi Kotani of the Laboratory of Immunology, Faculty of Pharmacy, Musashino University, Japan.

Method

● Preparation of template cDNA

Primary hepatocytes harvested from mice were subjected to various stimulation (IFN γ , siRNA), and their RNA were extracted. Total RNA (1000 ng) was reverse-transcribed into cDNA, and 1/25 volume of the reaction solution was used as a template. (cDNA derived from 40 ng of total RNA)

● PCR Reaction mixture compositions

〈KAPA HiFi〉

2xKAPA HiFi HotStart ReadyMix	12.5 μ L
Forward primer (10 μ M)	0.75 μ L
Reverse primer (10 μ M)	0.75 μ L
Template cDNA	2 μ L
PCR grade water	9 μ L
Total	25 μ L

〈Manufacturer To's High-fidelity enzyme〉

DNA polymerase	0.5 μ L
2mM dNTP	2.5 μ L
10 \times PCR buffer	2.5 μ L
25mM MgSO ₄	1 μ L
Forward primer (10 μ M)	0.75 μ L
Reverse primer (10 μ M)	0.75 μ L
Template cDNA	2 μ L
PCR grade water	15 μ L
Total	25 μ L

● Thermal cycle program

〈KAPA HiFi〉

94°C	2min
94°C	20sec
60°C	30sec
72°C	90sec
72°C	2min

] \times 35cycle

〈Manufacturer To's High-fidelity enzyme〉

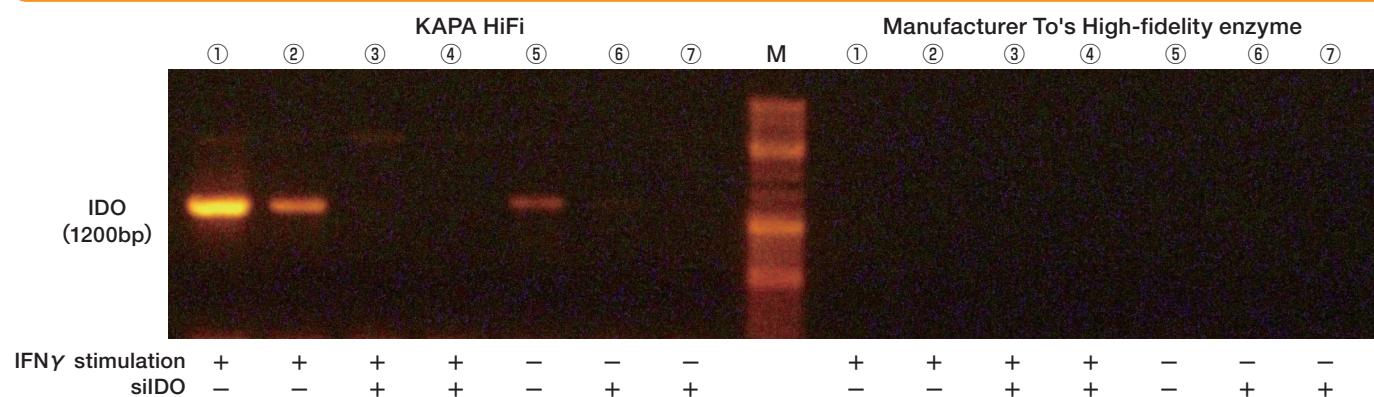
94°C	2min
94°C	20sec
60°C	30sec
68°C	90sec
68°C	2min

] \times 35cycle

● Amplicon size 1200 bp

● Thermal cycler : TaKaRa PCR Thermal Cycler Dice standard

Results



The effect of siRNA-mediated knockdown of IFN γ -induced IDO (indoleamine 2,3-dioxygenase) was examined using PCR. The KAPA HiFi HotStart ReadyMix could detect the band representing IFN γ -induced IDO and disappearance of the band demonstrating the occurrence of siRNA-mediated knockdown.

<Customer's comments>

I came across this product on the website and found it interesting, so I tried it. I never thought that the choice of enzyme would affect the quality of the target PCR product so much.