ENZYMES

KOD exo(-) DNA Polymerase/Anti-KOD Hotstart Antibody

ORIGIN ***E.coli*** **(Recombinant)**

CAT# KODND Range
EC# 2.7.7.7

ENZYME SPECIFICATION

Concentration/Activity*	2.5 U/µL
Endonuclease Activity	None detected
Nicking Activity	None detected

*One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTP into an acid insoluble material in 30 min at 75°C.

HOTSTART ANTIBODY SPECIFICATION

Source	Mouse Hybridoma
Inhibition Activity**	≥70%
ssEndonuclease Activity	None detected
dsEndonuclease Activity	None detected
5' to 3' Exonuclease Activity	None detected
3' to 5' Exonuclease Activity	None detected
Mouse Genomic DNA Contamination	None detected

**Percentage (%) of DNA polymerase activity surpressed when 1.0 µg of antibody is added to 1.25 units of KOD DNA polymerase at 37°C.

PRODUCT FORMAT

PART #	DESCRIPTION	CONTENT/FORMAT
KODND-109B	KOD exo(-) DNA Polymerase Kit	0.4mL (1,000U) KOD exo(-) DNA Polymerase 4mL 10x PCR Buffer for KOD exo(-)
KODND-119	KOD exo(-) DNA Polymerase 5KU	2mL KOD exo(-) DNA Polymerase
On Request	10x PCR Buffer for KOD exo(-)	20mL 10x PCR Buffer for KOD exo(-)
KAB2-101B	Anti-KOD Hotstart Antibody 8.2mg Kit	8.2mg Anti-KOD Antibody Dilution Buffer

SEKISUI DIAGNOSTICS
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ENZYME DESCRIPTION AND APPLICATION

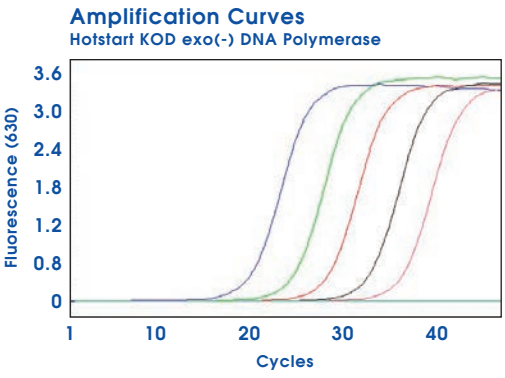
KOD DNA polymerase exhibits an excellent elongation capability (approx. 130 bases/sec) and strong 3′→5′ exonuclease activity (proofreading activity). However, its sensitivity and efficiency are subject to interference from the enzyme or template concentration during PCR, due to its strong proofreading activity. KOD exo(-) is a 3′→5′ exonuclease minus mutant developed based on KOD DNA polymerase from a hyperthermophilic Archaeon *Thermococcus kodakaraensis*.

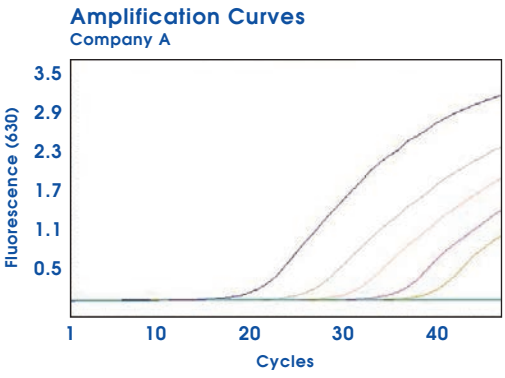
CHARACTERISTICS

Features:

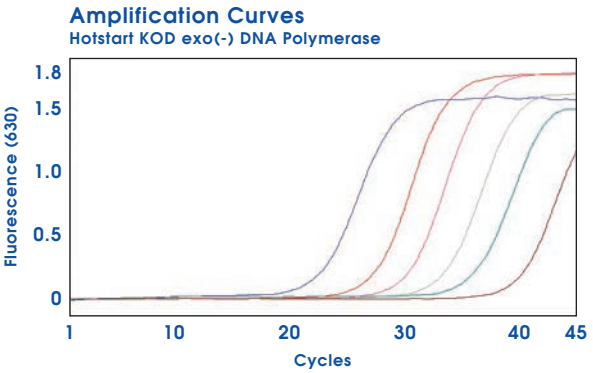
- **Excellent processivity & extension ability:** Extention time of the thermal cycle can be set <30 seconds / kb.
- **Great sensitivity & efficiency:** PCR sensitivity and efficiency are improved due to elimination of 3′-5′ exonuclease activity.
- **Hot start PCR:** anti-KOD DNA polymerase antibody for the polymerase activity is available.
- **Can be applied to the real-time PCR:** This enzyme can be applied to the real-time PCR assay, except for TaqMan assay due to lacking of the 5′-3′ exonuclease activity.
- A fast mode cycle can be applied.

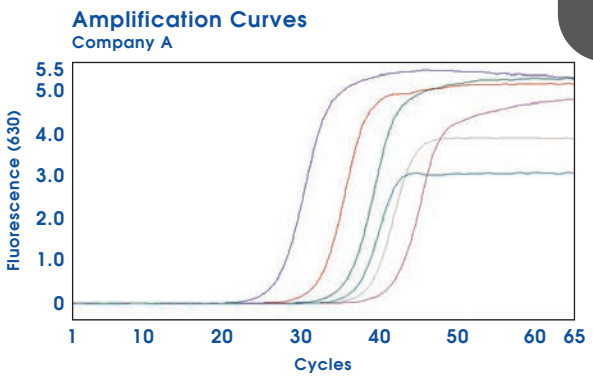
Application Data: Amplification of the β-actin gene (188 bp) was detected using serially diluted cDNA solutions (10n dilution) derived from 1 mg of human total RNA with real-time PCR (SYBR Green assay) using KOD exo(-)/Antibody and Company A's mix. KOD exo(-) showed greater sensitivity and efficiency than Company A's mix.





Application Data: Amplification of the β -actin gene (582 bp) was detected using serially diluted human genomic DNA (10n dilution: 50 ng-5 pg) with real-time PCR (SYBR Green assay) using KOD exo(-)/Antibody and Company A's mix. KOD exo(-) showed greater sensitivity and specificity than Company A's mix. The patterns of the melting curves indicate that the amplified products in the blank and 5 pg are primer dimers.





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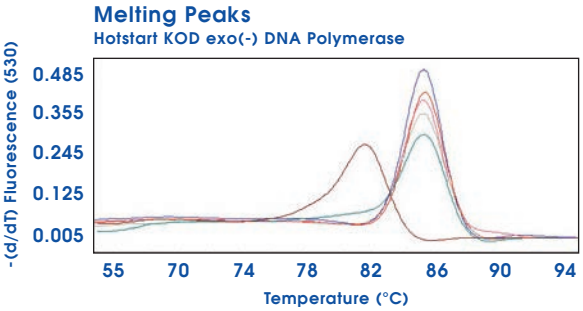
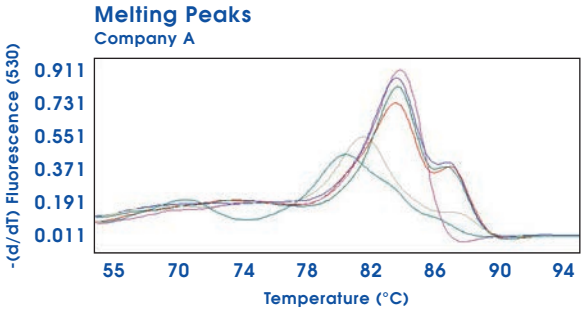
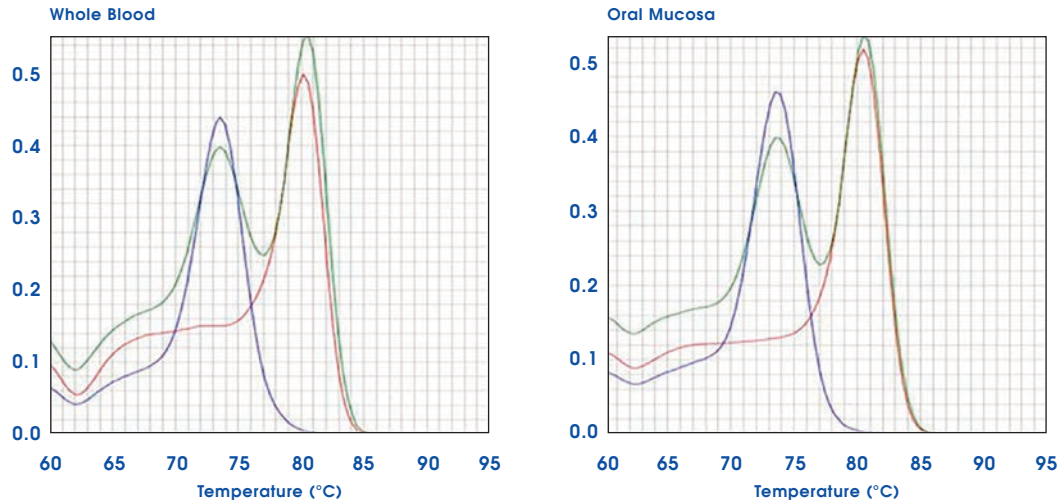




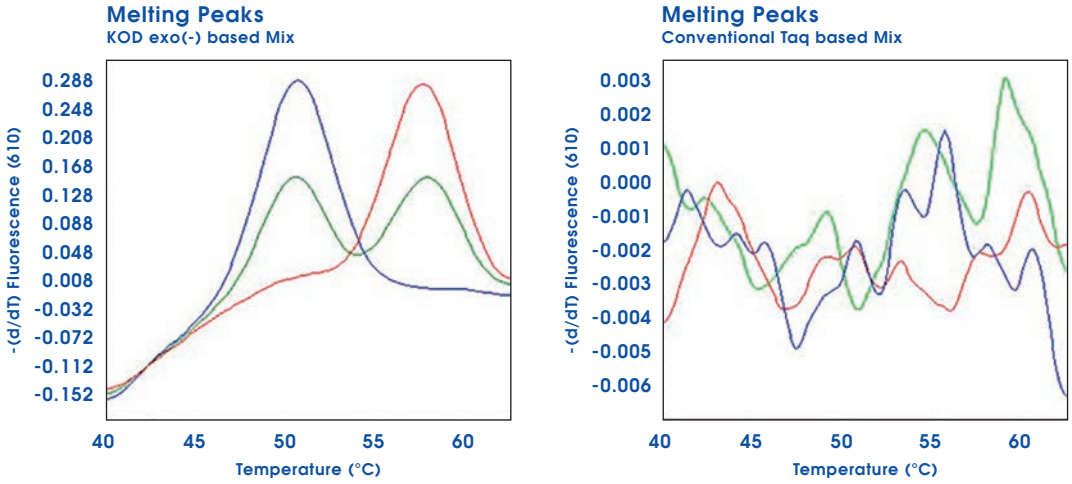
DETECTION OF SNPs

Detection of SNPs from crude specimens: Detection of single nucleotide polymorphisms (SNPs) in the human aldehyde dehydrogenase 2*(ALDH2)* gene using ASP-PCR. SNPs in *ALDH2* from the whole blood and oral mucosa specimens were detected using the allele-specific primers followed by melting curve analysis. In this experiment G-specific primer with GC-tail was used to increase the Tm of G-specific amplification.

The A/A, A/G, and G/G variants were detected in the crude specimen samples. As expected, peaks from both the 45 and 57 bp amplicons were detected by melting curve analysis at 73°C and 80°C, respectively.



Detection of SNPs using Hyb Probes: SNPs were detected from whole blood specimens using donor and acceptor probes bearing a fluorophore and acceptor at the 3′ and 5′ ends of the probe, respectively. The polymorphic site was designed in the donor probe. The two alleles were distinguished distinctly using the Hyb Probe method.



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