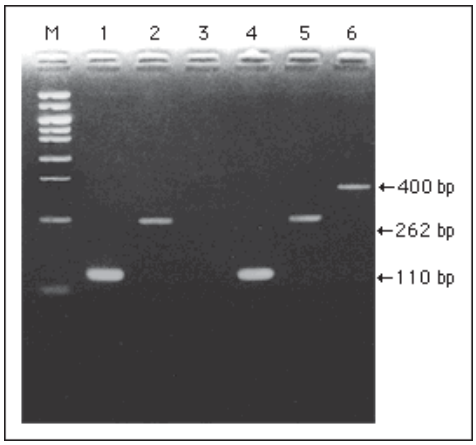
Application: Amplification of β-globin, K-ras 12, p53 (exon 5, exon 6) genes from 10-year-old paraffin sections

TaKaRa DEXPAT™ (DNA Extraction from Paraffin-embedded Tissue) (Cat.# 9091)

Genomic DNA was extracted from sections of 10-year-old paraffin-embedded colon cancer tissues using DEXPAT™ and used as a template for PCR to amplify fragments from the β-globin, K-ras 12 and p53 genes. To achieve accurate and highly sensitive PCR amplification, DNA extracted with DEXPAT™ is best used in conjunction with *TaKaRa Ex Taq*™ (Cat. # RR001)

A. Amplification of β-globin gene fragments (110, 262 and 400 bp):

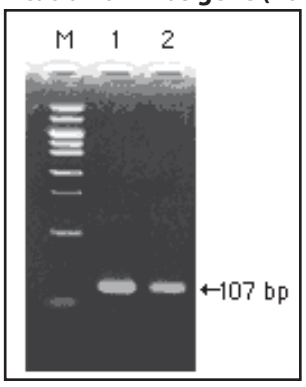


Lane M: pHY Marker (100 ng)
Lane 1-3: Amplification products using 5 µl of template DNA extracted using a conventional method
Lane 4-5: Amplification products using 5 µl of template DNA extracted using DEXPAT™
PCR volume: 50 µl
Polymerase: *TaKaRa Ex Taq*™

Thermal Cycling Conditions:

94°C, 30 sec.
54°C, 60 sec.
72°C, 60 sec.
↓ 35 cycles
72°C, 5 min.: 1 cycle

B. Amplification of K-ras gene (107 bp)

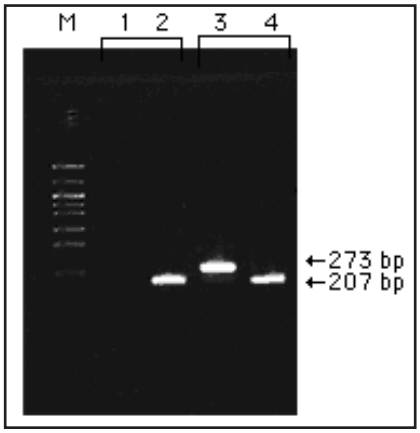


Lane M: pHY Marker (100 ng)
Lane 1-3: Amplification products using 5 µl of template DNA extracted using a conventional method
PCR volume: 50 µl
Polymerase: *TaKaRa Ex Taq*™

Thermal Cycling Conditions:

94°C, 30 sec.
54°C, 60 sec.
72°C, 60 sec.
↓ 35 cycles
72°C, 5 min.: 1 cycle

C. Amplification of p53 gene fragments from exons 5 (273 bp) and 6 (207 bp):



Lane M: pHY Marker (100 ng)
Lane 1 & 2: Amplification products from DNA extracted using a conventional method
Lane 3 & 4: Amplification products from DNA extracted using DEXPAT™
PCR volume: 50 µl
Polymerase: *TaKaRa Ex Taq*™

continued ...

Application: Amplification of β-globin, K-ras 12, p53 (exon 5, exon 6) genes from 10-year-old paraffin sections

TaKaRa DEXPAT™ (DNA Extraction from Paraffin-embedded Tissue) (Cat.# 9091)

(continued)

1st PCR thermal cycling conditions:

Template: 5 µl of template DNA extracted using DEXPAT™ or a conventional method
PCR: 50 µl
Polymerase: *TaKaRa Ex Taq™*

94°C, 1 min..
60°C, 1 min.
72°C, 2 min.
↓ — 30 cycles
72°C, 5 min.: 1 cycle

2nd PCR thermal cycling conditions:

Template: 1 µl of 1st PCR product
PCR: 50 µl
Polymerase: *TaKaRa Ex Taq™*

94°C, 30 sec.
60°C, 30 sec.
72°C, 60 sec.
↓ — 25 cycles
72°C, 5 min.: 1 cycle