Application: DNA extraction from normal and cancer tissues in a section on a histology slide

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

Loss of heterozygosity (LOH) in colon cancer tissue was assessed by PCR using microsatellite markers. DNA was extracted by DEXPAT™ from normal tissue and from neoplastic lesions of formalin-fixed, paraffin-embedded colorectal tumors. To achieve accurate and highly sensitive PCR amplification, DNA extracted with DEXPAT™ is best used in conjunction with *TaKaRa Ex Taq*™ (Cat.# RR001)

Protocol

1. Tissue sections (4.5 × 1.5 cm, 10 µm thick) were cut from paraffin-embedded tissue and mounted on a glass slide. One section was stained with hematoxylin and eosin to differentiate normal tissue from neoplastic tissue. The location of the neoplastic lesion (2.3 × 0.9 cm) was marked on the back side of the slides containing neighboring tissue sections.

2. The different tissues were recovered by the method shown in Figure 1. 500 µl of DEXPAT™ was added to each of two tubes and preheated at 100°C in a block heater. First, a small volume of preheated DEXPAT™ from a tube was placed on the neoplastic tissue identified by the markings on the slide. The colon cancer tissue was then scraped off the slide using the micropipette tip and the tissue along with the DEXPAT™ was collected into the micropipette and added to the corresponding tube containing the preheated DEXPAT™. The same protocol was used to collect normal tissue and add it into the second preheated tube.

3. The tubes were incubated at 100°C for ten minutes in a heat block and then centrifuged for ten minutes at 12,000 rpm at 4°C. Supernatants were collected in clean tubes and used as template DNA for PCR (Figure 2).

PCR reaction mixture:

10X *Ex Taq*™ Buffer	2.5µl
dNTP Mixture (2.5 mM ea.)	2.5 µl
Primer #1 (20 pmol/µl ea.)	0.25 µl
Primer #1 (20 pmol/µl ea.)	0.25 µl
DEXPAT™ DNA template	2.5 µl
TaKaRa Ex Taq™	0.125 µl
dH_2O	up to 25 µl

Thermal cycling conditions:

94°C, 90 sec.
↓
94°C, 30 sec.
54°C, 60 sec.
72°C, 60 sec. — 35 cycles
↓
72°C, 5 min.: 1 cycle

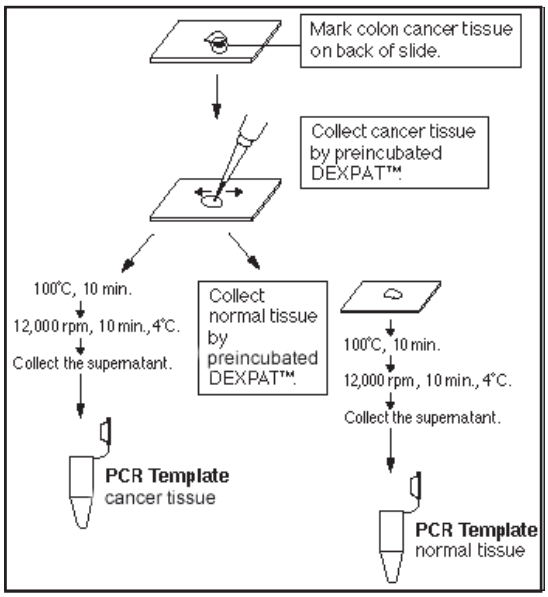

Figure 1. Flow chart of the protocol

continued ...

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(continued)

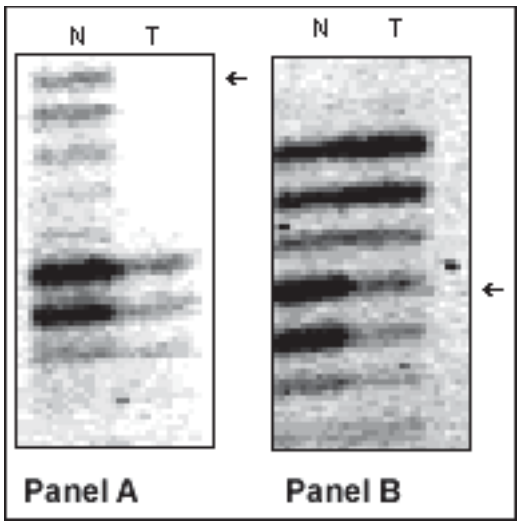

Figure 2. LOH analysis of tumor tissue using DNA extracted from histology slide tissues.

Panel A: LOH of the p53 target gene was assessed by PCR analysis using the TP53 microsatellite marker. Lane N: normal tissue. Lane T: tumor tissue.

Result: The absence of the p53-specific bands at the position marked by the arrow indicates 100% LOH for the p53 gene.

Panel B: LOH of the hMLH1 target gene was assessed by PCR analysis using the D3S1067 microsatellite marker. Lane N: normal tissue. Lane T: tumor tissue.

Result: The reduced intensity of the hMLH1-specific bands in the tumor tissue DNA at the position marked by the arrow indicates 81% LOH for the hMLH1 gene.

Conclusion:

LOH for the p53 and MLH1 genes in the tumor tissue was confirmed.