Cat. # 9091

For Research Use

TaKaRa

TaKaRa DEXPAT™

(DNA Extraction from Paraffin-embedded Tissue)

Product Manual

v201907Da

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I. Description

TaKaRa DEXPAT is a reagent designed for one-step extraction of DNA from paraffin-embedded tissues fixed with 10% formalin (3.5% formaldehyde). By using TaKaRa DEXPAT, the time for the preparation of PCR-ready DNA from paraffin-embedded tissue is dramatically reduced to 25 min from 2 - 3 days required for a conventional method. DEXPAT offers the following advantages;

1. No deparaffinizing step.
2. Only 10 min for extraction. (Total 25 min)
3. PCR-ready DNA (for <400 bp amplicon) is extracted.
4. No harmful component included.
5. Differential extraction of DNA from a tissue section on a histology slide increase the sensitivity and accuracy in detection of abnormalities.

TaKaRa DEXPAT utilizes ion exchange resin and surfactants, which is designed to optimize DNA extraction from paraffin-embedded tissue and PCR-ready DNA is extracted in the supernatant. Since DNA extracted from a paraffin-embedded tissue may be already degraded during formalin fixation or paraffin embedding, >400 bp DNA may not be amplified in PCR. The quality of extracted DNA depends on the condition of the embedded tissue.

This product is for DNA extraction from mammalian paraffin section to detect mammalian genes by PCR. It cannot be available for the detection of bacteria or fungi genes.

II. Components(100 reactions)

DEXPAT	10 ml x 5

III. Materials Required but not Provided

1. Micropipette and micropipette tips
2. Refrigerated microcentrifuge (12,000 rpm)
3. Heat block (capable of temperature settings up to 100℃)
4. 1.5 ml tube (One with a scale shall be convenient.)
5. Plastic gloves

IV. Storage

4℃

V. Note

1. Success of PCR using extracted DNA depends on the conditions for paraffin-embedding a tissue sample. In general, 10% formalin (3.5% formaldehyde) fixation for shorter than 3 days, is recommended. Also, acetone based AMeX fixation method[1)] has been reported for DNA analysis.

2. To avoid nuclease contamination, wear plastic gloves and all the equipment, such as tweezers and micropipet tips should be autoclaved before use.

3. When cutting sections from paraffin-embedded blocks, the microtome's blade and tweezers should be cleaned between each samples to avoid cross contamination of DNA. Use disinfectant (hydrogen peroxide type) and ethanol to clean the microtome's blade and tweezers. Further UV irradiation for 10 min is recommended.

4. Gently mix by rotation of DEXPAT bottle when adding to each tube, not to generate bubbles. Resin will be precipitated easily.

VI. Protocol

1. Prepare 5 μm (4 - 10 μm is available) thick paraffin-embedded tissue sections and add 1 - 3 tissue sections into microcentrifuge tube using sterilized tweezers. At least 6 mm x 6 mm size of tissue section should be prepared.

2. Gently mix by rotation of DEXPAT bottle. Add 0.5 ml (about 20 drops) of DEXPAT into the microcentrifuge tube. Mix bottle at each sampling to avoid resin downing.

3. Incubate the microcentrifuge tube at 100℃ for 10 min in heat block.

4. Immediately centrifuge the microcentrifuge tube at 12,000 rpm for 10 min at 4℃. (Figure 1) with the microcentrifuge precooled at 4 ℃.

5. Remove the paraffin thin layer to the tube wall and collect the supernatant using micropipette (Figure 2). Be sure not to withdraw the tissue debris and resin.

6. Use 5 μl of the supernatant as the template for 50 μl PCR reaction. The supernatant can be used less than 1/10 volume of PCR reaction. Excess amount of the supernatant may inhibit PCR reaction.

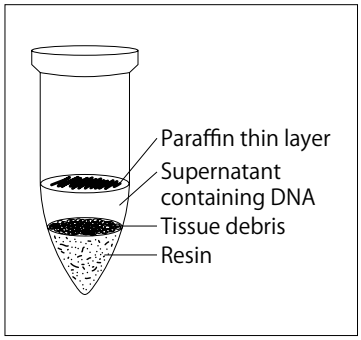


Figure 1

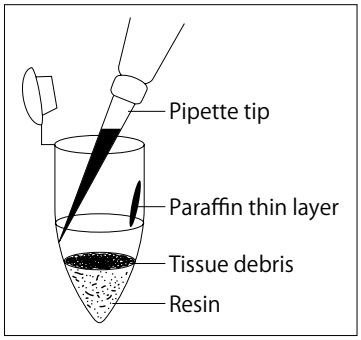


Figure 2

Schematic diagram

TaKaRa DEXPAT

Transfer 4 - 10 μm thick Paraffin-embedded tissue sections (1 - 3 pcs) into 1.5 ml tube
↓
Add 0.5 ml DEXPAT
↓
10 min at 100℃
↓
Centrifuge at 12,000 rpm for 10 min at 4℃
↓
Extracted DNA (Supernatant)
↓
PCR

Total: 25 min

Conventional Method[2)]

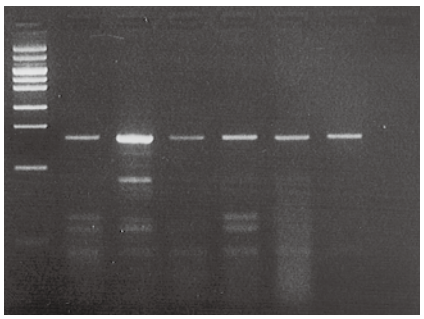
Paraffin-embedded tissue
↓
Deparaffinizing
↓
Dry up
↓
Protein removal step
↓
Phenol/Chloroform extraction
↓
Ethanol precipitation
↓
Dry up
↓
Dissolution
↓
Extracted DNA solution
↓
PCR

Total: 2 - 3 days

VII. Experimental Examples

A. Amplification of β-globin gene

DNA extracted from 6 paraffin sections using DEXPAT (each 10 μm, 2 pcs) were used as the templates for PCR. Extracted DNAs were stable at 4℃ for at least one month.



Lane M : pHY Marker (100 ng)

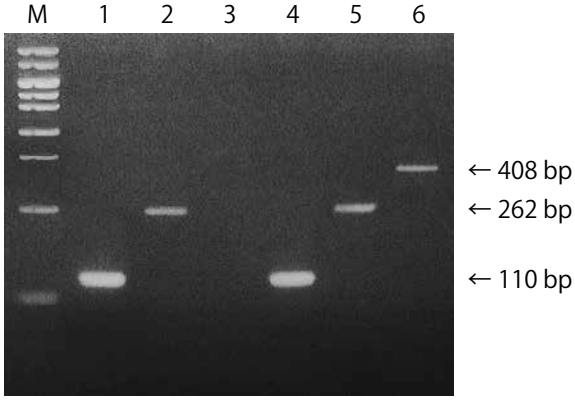
Template :
5 μl of extracted DNA using DEXPAT
PCR : 50 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :
94℃ 30 sec
54℃ 60 sec
72℃ 60 sec
] 35 cycles
↓
72℃ 5 min

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

DNA extracted from 10 year-old paraffin-embedded colon cancer tissues (10 μm, 2 pcs) using DEXPAT were used as the templates for PCR.

B-1) Amplification of β-globin genes (110, 262, 408 bp)



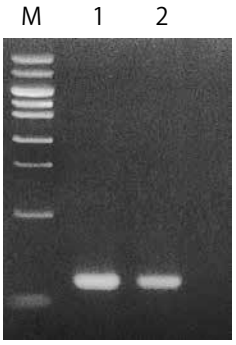
Lane M : pHY Marker (100 ng)

Template :
- Lane 1 - 3 :
  5 μl of DNA extracted with a conventional method
- Lane 4 - 6 :
  5 μl of DNA extracted using DEXPAT

PCR : 50 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :
94℃ 30 sec
54℃ 60 sec
72℃ 60 sec
] 35 cycles
↓
72℃ 5 min

B-2) Amplification of K-*ras* gene (107 bp)



Lane M: pHY Marker (100 ng)

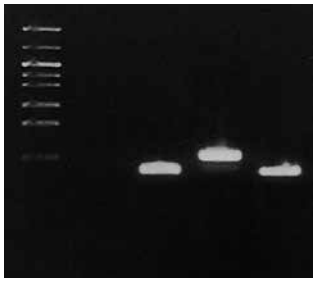
Template :
- Lane 1 :
  5 μl of DNA extracted with a conventional method
- Lane 2 :
  5 μl of DNA extracted using DEXPAT

PCR : 50 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :

94℃ 30 sec
54℃ 60 sec
72℃ 60 sec
] 35 cycles
↓
72℃ 5 min

B-3) Amplification of p53 exon 5 (273 bp), exon 6 (207 bp)



Lane M: pHY Marker (100 ng)

[1st PCR]
Template :
- Lane 1, 2 :
  5 μl of DNA extracted with a conventional method
- Lane 3, 4 :
  5 μl of DNA extracted using DEXPAT

PCR : 50 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :

94℃ 1 min
60℃ 1 min
72℃ 2 min
] 30 cycles
↓
72℃ 5 min

[2nd PCR]
Template : 5 μl of 1st PCR product
PCR : 50 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :

94℃ 30 sec
60℃ 60 sec
72℃ 60 sec
] 25 cycles
↓
72℃ 5 min

C. PCR amplification of DNA from frozen tissue sections on a slide glass

Samples

Paraffin embedded sections of human tonsil on a slide glass (5 μl thick, 25 mm x 12 mm)

Frozen sections of human tonsil on a slide glass (5 μl thick, 9 mm x 12 mm)

DNA Extraction

Pre-heat DEXPAT at 100℃ and put 500 μl DEXPAT on a tissue section. Scrape it by micropipette tip and transfer it into a microcentrifuge tube.

↓

10 min at 100℃

↓ Centrifuge at 12,000 rpm for 10 min at 4℃

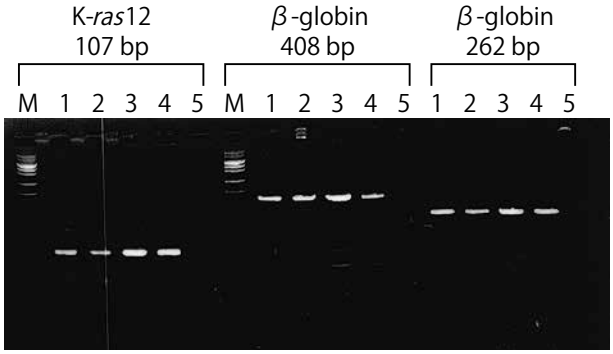
Supernatant

PCR Amplification

Target : K-*ras*12 (107 bp)
β-globin (408 bp, 262 bp)
Template : 1 μl or 2.5 μl of DEXPAT extract
PCR : 25 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition

94℃ 60 sec
↓
94℃ 30 sec ┐
54℃ 60 sec ├ 35 cycles
72℃ 60 sec ┘
↓
72℃ 5 min



3% Agarose, 5 μl apply

M : pHY marker
1 : 1 μl of extract from paraffin section
2 : 2.5 μl of extract from paraffin section
3 : 1 μl of extract from frozen section
4 : 2.5 μl of extract from frozen section
5 : No template

D. Differential extraction of DNA from tissue section on a histology slide

Analysis of LOH (loss of heterozygosity) in colon cancer was performed by PCR using microsatellite markers. DNA were extracted with DEXPAT from the normal and neoplastic tissue regions of the formalin-fixed, paraffin-embedded colorectal tumors.

Protocol

1. Tissue sections (4.5 x 1.5 cm) were cut from the tissue blocks and mounted on a slide glass. One of the sections was stained with hematoxylin and eosin to localize the normal and neoplastic tissue region. Neoplastic tissue region (2.3 x 0.9 cm) was marked on the back side of the next slide glass in a series of tissue section.
2. Transfer 500 μl of DEXPAT into a tube and preincubate at 100℃ in a heat block. Take a small portion of preincubated DEXPAT using micropipette. Use 1 ml micropipette tip and place on the marked colon cancer tissue position. Scrape off colon cancer tissue by micropipette tip. Collect the scraped colon cancer tissue with DEXPAT by micropipette and return into the DEXPAT tube. Following the same protocol, also collect normal tissue and add into an another DEXPAT tube.
3. Incubate the tubes at 100℃ for 10 min in a heat block. Centrifuge for 10 min at 12,000 rpm at 4℃. Collect the supernatant for the further PCR reaction.

PCR condition

・Reaction mixture :

10X *Ex Taq* buffer	2.5 μl	
dNTP Mixture	2.5 μl	(ea. 250 μM)
each primer	0.25 μl	(final 10 pmol)
Template DNA	2.5 μl	
TaKaRa Ex Taq	0.125 μl	
Sterile purified water	up to 25 μl	

・PCR condition:

94℃ 90 sec
↓
94℃ 30 sec ┐
54℃ 60 sec ├ 35 cycles
72℃ 60 sec ┘
↓
72℃ 5 min

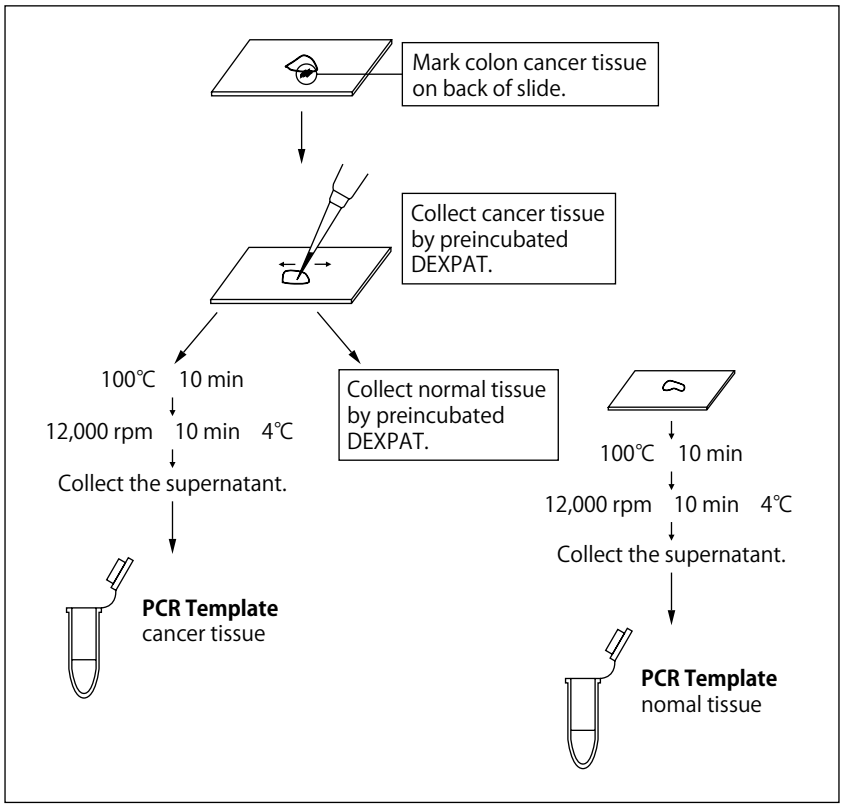
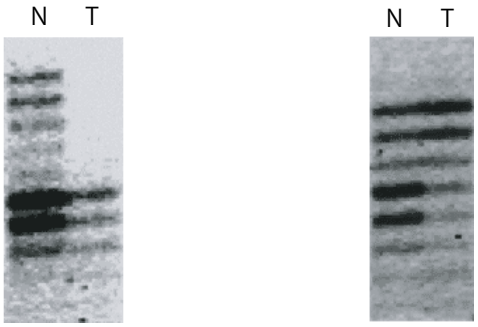


Figure 3. Flow chart of the protocol



Δ 100% LOH
Microsatellite marker : TP53
Target gene : p53

Δ 81% LOH
Microsatellite marker : D3S1067
Target gene : hMLH1 gene

Figure 4. LOH analysis using differential extracted DNA (N: normal, T: cancer tissue)

Result

LOH (Loss of heterozygosity) was confirmed.

VIII. Q & A

Q1 : Can RNA be extracted with DEXPAT ?
A1 : No. DEXPAT is used for DNA extraction only.

Q2 : Can DEXPAT be used for the DNA recovery from samples other than paraffin-embedded tissues ?
A2 : Yes. It is confirmed to be used for recovery from frozen tissues (See section VII. C). But it is not confirmed from the tissues after deparaffinization.

Q3 : Can extracted DNA be quantitated with absorbance ?
A3 : No. DEXPAT is designed only to extract DNA, not to purify DNA. So, extracted DNA cannot be quantitated correctly with absorbance.

Q4 : Can extracted DNA be analyzed by electrophoresis ?
A4 : The amount of extracted DNA is too small, so it is difficult to verify the DNA by electrophoresis.

Q5 : How long can the extracted DNA be stable?
A5 : It is stable for 3 months at 4℃, and for 1 year at -20℃.

Q6 : How much supernatant can be collected ?
A6 : Generally, 200 - 300 μl is collected.

Q7 : The supernatant cannot be collected.
A7 : The applied tissue section may be too big.
- Decrease the amount of tissue section or increase the volume of DEXPAT.
- Increase centrifugation speed (ex. 15,000 rpm).
- Mix well during heating to uniform the sample in DEXPAT.
- In the case that number of sample (microcentrifuge tube) is many for one centrifugation, the condition of the centrifugation (10 min, 4℃) is not enough to solidify the paraffin thin layer. In such a case, decrease the number of sample (tube) that is used for one centrifugation, or increase the centrifugation time.

Q8 : There is no band in PCR reaction using extracted DNA.
A8 : 1) Use the extracted DNA whose volume is 1/10 of PCR reaction solution, as the template for PCR. If more volume is required, change the buffer by ethanol precipitation.
2) DNA may be damaged markedly by fixing and embedding.
3) Some inhibitors may be contained. Purify the extracted DNA by ethanol precipitation＊.

＊ DNA Purification by ethanol precipitation
1. Estimate the volume of extracted DNA.
2. Add 1/10 volume of 3M sodium acetate.
3. Add 2.5 volumes of ethanol (or add same volume of isopropanol).
4. Mix uniformly by converting tubes.
5. Leave the tubes at -20℃ for 30 min - 1 hours.
6. Centrifuge at 12,000 rpm for 10 min at 4℃.
7. Remove the supernatant, and add 1 ml of 70% ethanol.
8. Centrifuge at 12,000 rpm for 10 min at 4℃.
9. Remove the supernatant, Dry the precipitate.
10. Dissolve the precipitate in 20 - 25 μl of the appropriate buffer (ex. TE buffer).

IX. References

1) Sato Y, *et al. Am J Pathol*. (1990)**136**: 267.
2) Goelz S E, *et al. Biochem Biophys Res Commun*. (1985)**130**: 118-126.

X. Related Products

TaKaRa Ex Taq® (Cat. #RR001)

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