TaKaRa DEXPAT Reagent FAQs

Cat.# 9091

Can DEXPAT reagent be used to extract DNA from tissue fixed on a slide?

DEXPAT cannot be used to extract DNA if the tissue may slip off the slide or a substance such as xylene has been used to peel the tissue off the slide. When extracting DNA from a slide, use the site-specific extraction method. However, be careful when extracting DNA from tissue that has been stained previously because the PCR reaction will be inhibited by the staining agent.

How long can DNA extracted with DEXPAT reagent be stored?

DNA extracted with DEXPAT reagent has been stored for up to three months at 4°C and up to one year at –20°C.

If the extracted sample contains a lot of tissue residue and a clean extraction supernatant cannot be obtained, what can be done to improve the purification?

The residue is thought to be caused by excess DNA in the extraction. Reducing the amount of DNA or increasing the amount of DEXPAT to 1 ml can improve the outcome. In addition, increasing the speed of the centrifugation to 15,000 RPM may allow the supernatant to be removed cleanly. Taking care to ensure that the sample tube cap remains in place (e.g., by using a screw-top tube or adding a lid lock device to a snap-top microfuge tube), agitate the sample gently during the boiling step to create a homogenous mixture of sample and the DEXPAT reagent.

Often, a thin film of paraffin may form above the top layer after the centrifugation step (12,000 rpm, 10 minutes, 4°C). After centrifugation, this thin paraffin film should be carefully moved to the side of the sample tube with a pipet tip so that the layer of DNA-containing supernatant underneath can be removed. However, if the tubes have warmed while processing multiple samples, the paraffin layer may be fragile and difficult to manipulate. In such cases, either spin the samples for a longer period or reduce the number of tubes being spun at one time so that the samples will be adequately cooled as they are centrifuged.

When I ran a PCR reaction using DEXPAT-extracted DNA, the target band yields were low. How can I improve my PCR results?

A) Ensure that the maximum volume of the DNA solution used in the PCR reaction is 1/10 the final reaction volume. If more template DNA is needed, collect the DNA by ethanol precipitation and resuspend it in fresh buffer compatible with PCR, e.g. 1X PCR reaction buffer.

B) The DNA may be fragmented. DNA is thought to undergo considerable damage due to the fixation and paraffin-embedding process.

C) The sample may contain a PCR-inhibiting agent(s). Purify the extracted DNA by ethanol precipitation and try performing PCR again.

D) It is possible that the bands cannot be detected because the amount of DNA contained in the processed sample is too low. Try to concentrate the extracted DNA by ethanol precipitation using the following method:

1. Estimate the volume of the extracted DNA solution.
2. Add a volume of 3M sodium acetate that is 1/10 the sample volume.
3. Add a volume of ethanol that is 2.5 times the sample volume, or add an amount of isopropanol equivalent to the sample volume.
4. Invert several times to mix thoroughly.
5. Let sit at –20° for 30 minutes to one hour.
6. Centrifuge at 12,000 × *g* for 10-15 minutes at 4°.
7. Remove the supernatant and add 1 ml of 70% ethanol.
8. Centrifuge at 12,000 × *g* for 10-15 minutes at 4°.
9. Carefully remove and discard the supernatant, and air-dry the sample.
10. Dissolve in an appropriate volume of TE or other buffer. (Dissolving in 20-25 µl buffer will result in an approximately 10-fold increase in concentration.)

Can DEXPAT reagent be used to extract RNA?

DEXPAT reagent can be used for extracting DNA only; it cannot be used to extract RNA.

Can DEXPAT reagent be used for extracting DNA from anything other than paraffin-embedded tissue samples?

We have confirmed that it can be used with frozen tissue samples. However, we have not verified that it can be used with deparaffinized tissue samples.

Can the extracted DNA be quantified with a spectrophotometer?

No. Because DEXPAT is a DNA extraction kit and not a DNA purification kit, the DNA cannot be quantified by UV absorption on a spectrophotometer.

Can I verify the yield of the extracted DNA by electrophoresis?

Possibly, but very often the DNA cannot be verified by electrophoresis as the amount recovered is very small, depending on the tissue sample.

How much of the aqueous layer can be typically recovered?

Usually 200-300 µl can be collected.