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General considerations for the storage of sample material prior to DNA purification*

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Different starting materials have different characteristics that affect DNA purification, particularly sample disruption and lysis, and removal of specific cell constituents. In addition, the quality of the sample and the way it was stored affect the quality and yield of the purified DNA. Tissue damage can result in degradation of DNA. Since many tissues cannot always be processed immediately after harvesting, the conditions under which the tissue is stored must preserve the integrity of the DNA. The following are general considerations for the storage of specific sample materials, in order to obtain high-quality DNA.

Storage of animal and human tissue

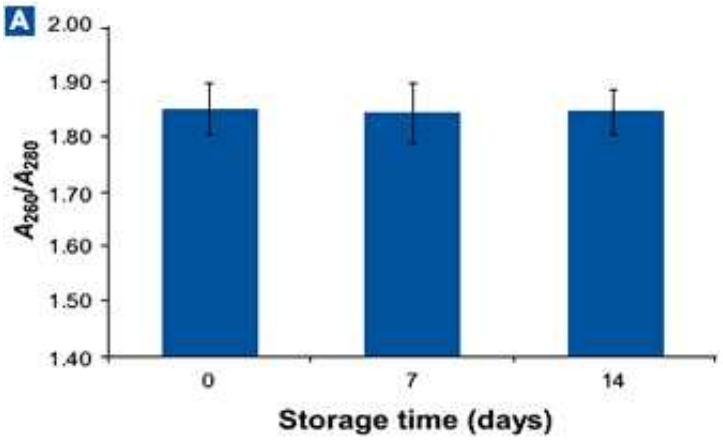
Freshly harvested tissue can be immediately frozen and stored at –20°C, –80°C, or in liquid nitrogen. Lysed tissue samples can be stored in a suitable lysis buffer for several months at ambient temperature.

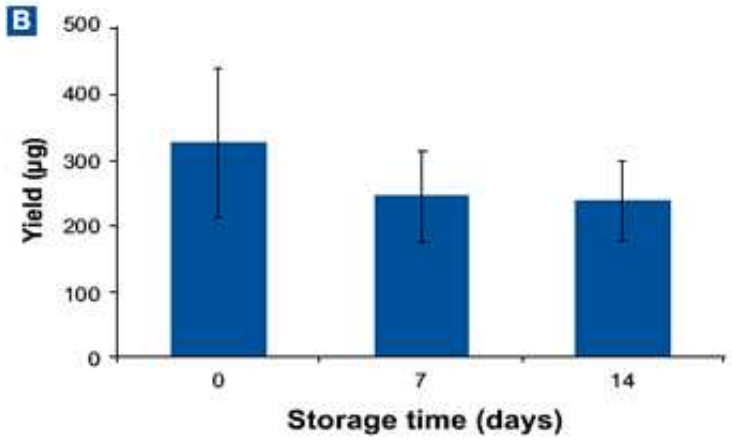
Animal and human tissues can also be fixed for storage. Fixatives that cause cross-linking, such as osmic acid, are not recommended if DNA will be purified from the tissue. Fixatives such as alcohol and formalin are recommended; however, long-term storage of tissues in formalin will result in chemical modification of the DNA. Protocols for the purification of DNA from formalin-fixed tissue or paraffin-embedded tissue are available in the *DNeasy Tissue Handbook*. Furthermore, QIAGEN supplementary protocols for automated DNA purification from paraffin-embedded tissues are available for the BioRobot EZ1 and BioRobot M48 workstations.

Storage of blood samples

An anticoagulant should be added to blood samples that will be stored. For example, blood samples treated with heparin or EDTA can be stored at 2–8°C for a few days, or at –20°C or –80°C for a few weeks. Alternatively, blood samples can be treated with ACD Solution B (0.48% citric acid, 1.32% sodium citrate, 1.47% glucose; use 1 ml per 6 ml blood; [1]) and stored for at least 5 days at 2–8°C or 1 month at –20°C. For long-term storage, blood nuclei can be prepared and stored at –20°C. The PreAnalytiX PAXgene Blood DNA System offers a complete solution for blood collection and stabilization and the subsequent purification of genomic DNA. The PAXgene Blood DNA Kit provides rapid (1 hour) purification of DNA from up to 8.5 ml of blood. Blood samples collected using PAXgene Blood DNA Tubes can be stored at room temperature (15–25°C) for up to 14 days, or at 2–8°C for up to 28 days.

Average Purity and Yield of DNA after Storage in PAXgene Blood DNA Tubes at 25ºC for up to 14 days





A *Average purity and* B *Average yield of DNA purified from whole blood samples from 70 healthy donors. Data from reference 2.*

QIAamp DNA Blood Kits are recommended for fast and easy purification of DNA from fresh and frozen whole blood and blood that has been treated with an anticoagulant.

In addition, DNA can be purified from dried blood that has been spotted onto filter paper (see below). QIAGEN offers protocols for purification of DNA from dried blood using the QIAamp 96 DNA Blood Kit, the EZ1 DNA Tissue Kit, or the MagAttract DNA Mini M48 Kit.

Storage of clinical samples

Most biological fluids (e.g., plasma, serum, and urine) and stool samples can be stored at 2–8°C for several hours. Freezing at –20°C or –80°C is recommended for long-term storage. Swabs can be stored dry at room temperature. For applications requiring only small amounts of DNA, such as expression profiling and genotyping, it is possible to extract DNA from stool samples that are at least 91 days old using the QIAamp DNA Stool Kit (3). Furthermore, QIAGEN offers a specific protocol for the purification of DNA from formalin-preserved stool samples using the QIAamp DNA Stool Mini Kit.

Filter-paper–based storage methods

Use of filter paper impregnated with various compounds for DNA stabilization and purification provides a convenient way to store dried biological samples and then isolate DNA from the samples for use in PCR (4). The filter paper contains compounds that lyse biological samples and bind nucleic acids, as well as compounds that kill microorganisms and inhibit non-microbial degradation of DNA (e.g., oxidation). The sample is simply spotted onto the paper and dried, and then the bound DNA is purified by a simple washing procedure. Depending on the type of filter paper used, purified nucleic acids either remain bound to the filter paper or are eluted before use. Dried samples can also be stored at room temperature for many years without loss in genomic DNA integrity.

Filter-paper methods have the advantages of a simple sample collection and storage process, as well as a fast, single-tube DNA purification procedure. In addition, processing of the stored samples can be automated. However, the range of applications in which the DNA can be used is limited mainly to PCR.

Storage of plant and fungal samples

Fresh leaves and needles from most species can be stored for up to 24 hours at 4°C without affecting DNA quality or yield. In general, samples that will be stored for longer than 24 hours should be frozen and stored at –80°C. However, some samples (e.g., tree buds) can be stored for several days at 4°C. Tissues stored at 4°C should be kept in a closed container to prevent dehydration. Large samples (e.g., branches) can be stored in a plastic bag containing a wet paper towel.

If it is not practical to store frozen plant samples, a number of methods are available for drying plant tissue, for example, silica gel, food dehydrators, or lyophilizers (5). To prevent DNA degradation, material should be completely desiccated in less than 24 hours. Dried samples should be kept in the dark at room temperature under desiccating or hermetic conditions for long-term storage (6). Depending on how the sample was handled, the DNA in herbarium and

forensic samples may be degraded. Disrupted plant material can be stored in a suitable lysis buffer at room temperature for several months.Fungal mycelium should be harvested directly from a culture dish or liquid culture. For liquid cultures, the cells should be pelleted by centrifugation and the supernatant removed before DNA purification or storage. Harvested samples can be either directly frozen or freeze dried, and stored at –80°C.

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