QIAsafe[™] DNA Tube and 96-Well Plate Handbook

For room temperature storage of DNA This product contains DNA SampleMatrix® technology developed by Biomatrica, Inc.



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Kit Contents

QIAsafe DNA	Tubes (50) 96-Well Plate	
Catalog no.	159104	159112
Number of samples	50	10 x 96
QIAsafe DNA Tubes	50	_
QIAsafe DNA 96-Well Plates	-	10
QIAsafe Seals	_	10
Handbook	1	1

Storage

QIAsafe DNA matrix is a novel formulation for safe, stable, and convenient storage of DNA at room temperature (15–25°C). QIAsafe DNA Tubes and 96-Well Plates should be stored dry in their original unopened packaging.

QlAsafe DNA Tubes and 96-Well Plates are supplied with moisture-barrier foil packages. These allow storage even in uncontrolled environments with elevated relative humidity above 50%. The packaging provided with the product is designed for proper storage using the resealable closure. Open the packages just before use

Note: Storage of opened QIAsafe DNA Tubes or 96-Well Plates at relative humidity above 50% for extended periods of time will reduce product performance and sample protection. Climate-controlled laboratory environments and buildings are normally maintained at 40–50% relative humidity levels.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAsafe DNA Tubes and QIAsafe DNA 96-Well Plates is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

QIAsafe DNA Tubes and 96-Well Plates are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit <u>www.qiagen.com</u>).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAsafe DNA Tubes, QIAsafe DNA 96-Well Plates, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support center at <u>www.qiagen.com/goto/TechSupportCenter</u> or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit <u>www.qiagen.com</u>).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at <u>www.qiagen.com/Support/MSDS.aspx</u> where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

QIAsafe DNA Tubes and 96-Well Plates provide innovative technology for biological sample storage at room temperature. Stabilizing DNA at room temperature saves on refrigeration costs and enables easy transportation and storage.

QlAsafe technology, developed by Biomatrica, Inc., was designed by combining extremophile biology and synthetic chemistry to create a novel dissolvable matrix optimally formulated for long-term ambient temperature storage and shipping of DNA, including genomic DNA, plasmids, bacterial artificial chromosomes (BACs), PCR products, and oligonucleotides.

Sample recovery from the QIAsafe matrix is as easy as "just add water". Rehydrated samples are ready for immediate use, without the need for further purification. Downstream applications using recovered samples include transformation, transfection, restriction enzyme analysis, cloning, sequencing, PCR, quantitative PCR, and microarray analysis. Samples stored dry in the QIAsafe matrix are ready for convenient shipping at ambient temperatures, even over extended transit times.

Principle and procedure

The QIAsafe matrix is a mixture of dissolvable compounds that stabilizes DNA at room temperature. The synthetic chemical matrix formulation is based upon the natural principles of anhydrobiosis. Anhydrobiosis, meaning "life without water", is a biological mechanism employed by some multicellular organisms that enables their survival in a dry state for periods over 100 years.* During these extended dry periods, proteins, DNA, membranes, and cellular systems are protected and can be revived by rehydration.

The QIAsafe matrix forms a protective seal around DNA as it dries, effectively "shrinkwrapping" the sample in a protective coating. Drying can occur at ambient temperatures with no need for special equipment. Stored dry at ambient temperatures, the protected DNA can be safely stored for extended time periods. DNA samples can be recovered from QIAsafe matrix through simple rehydration ("just add water") and are ready for immediate use, without the need for further purification.

^{*} Crowe, J.H., Carpenter, J.F., and Crowe, L.M. (1998) The role of vitrification in anhydrobiosis. Annu. Rev. Physiol. **60**, 73.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Water, TE buffer, or other aqueous buffer for downstream applications
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing crosscontamination are recommended)
- Recommended: Laminar flow hood or, for samples >20 µl, a vacuum concentrator for drying

Important Notes Types of DNA

All types of DNA can be stored in QIAsafe matrix, including genomic DNA, plasmids, oligonucleotides, PCR products, bacterial artificial chromosomes (BACs), and DNA from complex sources (e.g., forensic or genetic identity DNA samples).

Purification of DNA

DNA purified using most standard molecular biology techniques and/or commercially available kits is compatible with storage in QIAsafe DNA Tubes or 96-Well Plates. For optimal protection and sample recovery, DNA should be purified of any contaminating DNase activity. Purified DNA should be resuspended in water, TE buffer (10 mM Tris·Cl, 1 mM EDTA), or any QIAGEN DNA elution buffer prior to application into QIAsafe matrix.

QIAGEN provides a wide range of kits for manual and automated purification of DNA, depending on sample type and throughput needs. Use the ProductFinder at <u>www.qiagen.com/products/productfinder</u> to find the best QIAGEN product for your application.

Determining yield

The concentration of the DNA sample should be determined prior to sample application into QIAsafe DNA Tubes or 96-Well Plates. Although not essential, applying a known amount of DNA into the QIAsafe matrix for storage can facilitate sample retrieval and subsequent applications.

Drying of samples

Samples applied to wells or tubes containing QIAsafe matrix must be dried completely for optimal storage at room temperature. Sample volumes of up to 20 µl can be applied to a QIAsafe DNA Tube or well of a QIAsafe 96-Well Plate and dried at ambient temperatures on the lab bench or in a laminar flow hood (recommended). Larger sample volumes may require use of a vacuum concentrator for complete drying.

For optimal protection in QIAsafe matrix, do not apply more than a total of 30 µg of DNA per well or tube in a maximum volume of 20 µl. For oligonucleotides, we recommend storage of 20 µl aliquots, with a concentration of $\leq 100 \mu$ M per oligo (2 nmol of each oligo). For larger volumes, a vacuum concentrator may be required for efficient drying.

Sample recovery

To recover samples stored dry in QIAsafe matrix, just add water. Samples are ready for immediate use in all downstream applications. It is not necessary to further purify rehydrated samples. Aqueous solutions such as TE buffer (10 mM Tris·Cl, 1 mM EDTA), PCR reaction buffers, restriction enzyme buffers, and transfection reagents are also compatible with recovery of samples from QIAsafe matrix.

Protocol: Sample Drying and Storage

QIAsafe matrix is formulated so that upon application of liquid samples, the matrix dissolves and forms a protective coating around the DNA. The sample must then be completely dried for maximum protection and stability during storage at ambient temperatures.

Important point before starting

- Read "Important Notes", pages 9–10.
- If you want to determine the DNA concentration at a later date using a spectrophotometer, keep an unused QIAsafe DNA Tube or well of a QIAsafe 96-Well Plate to generate a blank. See Appendix A, page 17.

Things to do before starting

Purify DNA and make sure that it is dissolved in water, TE buffer, or a QIAGEN elution buffer (see "Purification of DNA", page 9).

Procedure

- 1. Determine the amount of purified DNA in the sample, and calculate the amount to be applied into QIAsafe DNA Tubes or wells of a QIAsafe 96-Well Plate.
- 2. Remove the seal or cap and gently apply the sample into the center of the vessel containing QIAsafe matrix.

QIAsafe matrix is supplied as a coating on the bottom of each well or tube. An aqueous sample will rehydrate the QIAsafe matrix within minutes.

Note: The final volume of the sample applied to each well should be $\leq 20 \ \mu$ l. For larger volumes, a vacuum concentrator is required to ensure complete drying. See Table 2 on page 12 for drying times.

3. Mix the sample thoroughly with gentle pipetting. Avoid forming air bubbles.

4. Dry the uncovered sample completely at room temperature (15–25°C).

We recommend using a laminar flow hood or drying under a vacuum to ensure complete drying. Recommended drying times are given in Tables 1 and 2 on page 12.

Note: Drying should occur at 15–25°C with relative humidity below 50%. Relative humidity above 50% for extended periods of time will reduce product performance and sample protection. Climate-controlled laboratory environments and buildings are normally maintained at 40–50% relative humidity levels. We recommend drying under a vacuum if conditions exceed these parameters.

Table 1. Drying times in laminar flow hood

Sample volume (µl)	Approximate drying time (h)
5	2
5–10	6
10–20	10–12

Table 2. Drying times in a vacuum concentrator

Sample volume (µl)	Approximate drying time (h)
10–20	0.5
20–30	1
30–50*	1.5
50–100*	2

* QIAsafe matrix is designed for optimal protection and recovery of sample volumes of \leq 20 µl.

5. Cover samples after drying and store at room temperature.

After complete sample drying, plates should be resealed with the QIAsafe Seals provided with the QIAsafe DNA 96-Well Plates. The QIAsafe Seal will provide an additional moisture barrier to protect the sample. QIAsafe DNA Tubes can be closed using the cap supplied with the tube.

Samples properly sealed can be stored at ambient temperature with relative humidity below 50%. Relative humidity above 50% for extended periods of time will reduce product performance and sample protection in QIAsafe DNA Tubes and 96-Well Plates. Climate-controlled laboratory environments and buildings are normally maintained at 40–50% relative humidity levels.

For storage in uncontrolled environments with relative humidity >50%, store the sealed QIAsafe DNA Tubes and 96-Well Plates in the moisture-barrier foil packages included with the product. Seal the packaging using the resealable closure.

Protocol: Sample Recovery

DNA stored inQIAsafe DNA Tubes or 96-Well Plates can be recovered by adding water or aqueous buffer. Samples are ready for downstream applications without the need for further purification.

Procedure

 Add 10–100 µl of water or aqueous buffer directly to the dried sample in a QIAsafe DNA Tube or well of a QIAsafe 96-Well Plate.

Individual wells in a plate can be opened by puncturing the aluminum foil seal with a pipette tip or razor blade.

Samples may be rehydrated directly with aqueous buffers used for downstream applications such as restriction enzyme buffers, PCR buffers, and transfection reagents.

- 2. Incubate at room temperature (15–25°C) for 15 min to allow complete rehydration.
- 3. Mix the sample by gently pipetting up and down to resuspend the sample. Avoid forming bubbles while pipetting.

The rehydrated sample is now ready for use directly in downstream applications. Real-time PCR analysis using SYBR[®] Green or sequence-specific probes should be performed using the guidelines given in Appendix B, page 18.

If further purification is desired, samples can be purified using the QIAamp® DNA Micro Kit for genomic DNA or the MinElute® PCR Purification Kit for plasmid DNA.

See Appendix A, page 17, for UV-spectrophotometric determination of DNA concentration and yield (i.e., A_{260} measurement). Since the spectrophotometer must be zeroed against a QIAsafe matrix blank, rehydrate an unused well or tube with the same buffer used to rehydrate the DNA sample.

4. Store unused rehydrated samples at 2–8°C or room temperature for up to 10 days.

Rehydrated samples contain QIAsafe matrix and can be re-dried without loss of efficient sample stabilization. We do not recommend repeating the rehydration–drying process more than 3 times.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: <u>www.qiagen.com/FAQ/FAQList.aspx</u>. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit <u>www.qiagen.com</u>).

Low yield of rehydrated DNA			
a)	DNases in sample	Purified DNA safely stored in QIAsafe matrix will not degrade unless it is contaminated by considerable quantities of DNases. Ensure that samples are purified so that they are DNase-free before applying to the QIAsafe matrix.	
b)	Low amount of DNA in initial sample	Before applying the sample, measure the concentration and record the amount of DNA added. Identical samples can be rehydrated separately and combined.	
c)	Sample not properly applied	Make sure to apply the sample to the center of the QIAsafe DNA Tube or well of a QIAsafe DNA 96-Well Plate so that it does not stick to the sides of the tube or well.	
d)	Too small a volume used for rehydration	Samples should be rehydrated using 10–100 µl water, TE buffer, or other aqueous buffer for downstream applications. Use of <10 µl can inhibit complete rehydration and decrease recovery.	
e)	Reduced rehydration time	A minimum rehydration time of 15 min is important for complete recovery of stored DNA. A shorter time of 5 min, with mixing, can be used, but the sample yield will be slightly reduced.	

Comments and suggestions

f)	Increased rehydration time	Maximum rehydration time should not exceed 1 h. Keep QIAsafe DNA Tubes and 96-Well Plates covered with the cap or foil seal during rehydration to avoid contamination and evaporation.
g)	Reduced volume of rehydrated sample	Rehydration of the QIAsafe matrix may cause a reduction in sample volume, especially when smaller volumes are used for rehydration (<20 µl). Minimal loss of recovered sample volume does not affect the stability or performance of DNA in downstream applications. With smaller rehydration volumes, a 10% loss in volume can be included for adjustment (e.g., add 11 µl of water to ensure recovery of 10 µl of rehydrated sample).
Rec	overed DNA is degraded	
a)	Samples not properly dried	Samples must be completely dried before storage. See Tables 1 and 2, page 12, for drying guidelines.
b)	DNases in sample	Purified DNA safely stored in QIAsafe matrix will not degrade unless it is contaminated by considerable quantities of DNases. Ensure that samples are purified so that they are DNase-free before applying to the QIAsafe matrix.
c)	Storage in humid environment	DNA in QIAsafe matrix must be stored at room temperature (15–25°C) with relative humidity below 50%. Properly sealed samples can be stored at lower temperatures (e.g., 2–8°C, -15 to -30°C, or -80°C) without affecting product performance.
		For storage in uncontrolled environments with relative humidity >50%, store the sealed QIAsafe DNA Tubes and 96-Well Plates in the moisture-barrier foil packages included with the product. Seal the packaging using the resealable closure.

Comments and suggestions

Cross-contamination between samples			
a)	Improper handling	Use pipet tips with aerosol barriers to prevent cross-contamination. Always wear gloves when handling QIAsafe DNA Tubes and 96-Well Plates. Change gloves frequently and keep QIAsafe DNA Tubes and 96-Well Plates closed whenever possible.	
b)	QIAsafe DNA 96-Well Plates: Too much buffer used for rehydration	Use of large volumes (>100 μ l) for rehydration may cause overflow of wells and cross-contamination between wells. Samples can be rehydrated in the recommended volumes and then transferred to a larger vessel and brought up to the desired volume.	
Unused QIAsafe matrix faded or changes color			
	Color of unused QIAsafe matrix changes with storage	Fading or color change of QIAsafe matrix does not affect protective properties. Proceed with sample application drving	

Proceed with sample application, drying, storage, and rehydration according to the protocols.

Appendix A: Determination of Yield and Purity of DNA

Determination of yield and purity

DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Water should be used as diluent when measuring DNA concentration since the relationship between absorbance and concentration is based on extinction coefficients calculated for nucleic acids in water.*

The UV spectrophotometer must be calibrated against an aliquot of QIAsafe matrix for accurate A_{260} readings. Rehydrate an unused QIAsafe DNA Tube or well of a QIAsafe DNA 96-Well Plate containing only QIAsafe matrix (no DNA) using the same volumes (including dilutions) as was performed for sample preparation.

Measure the absorbance at 260 nm or scan absorbance from 220–330 nm (a scan will show if there are other factors affecting absorbance at 260 nm; for instance, absorbance at 325 nm would indicate contamination by particulate matter or a dirty cuvette). An A_{260} value of 1 (with a 1 cm detection path) corresponds to 50 µg DNA per milliliter water for double-stranded DNA and 38 µg DNA per milliliter water for single-stranded DNA and RNA are measured with a spectrophotometer at 260 nm; to measure only DNA in a mixture of DNA and RNA, a fluorimeter must be used.

An example of the calculations involved in DNA quantification is shown below.

Volume of DNA sample = $100 \ \mu l$

Dilution = 20 μ l of DNA sample + 180 μ l distilled water

(1/10 dilution)

Measure absorbance of diluted sample in a 0.2 ml cuvette

 $A_{260} = 0.2$

Concentration of DNA sample = 50 μ g/ml x A_{260} x dilution factor

- $= 50 \ \mu g/ml \ x \ 0.2 \ x \ 10$
- = 100 µg/ml

Total amount = concentration x volume of sample in milliliters

- = 100 µg/ml x 0.1 ml
- = 10 µg DNA

^{*} Wilfinger, W.W., Mackey, M., and Chomcynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of DNA with respect to contaminants that absorb UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination. For accurate values, we recommend measuring absorbance in 10 mM Tris-Cl, pH 7.5, in which pure DNA has an A_{260}/A_{280} ratio of 1.8–2.0. Always be sure to calibrate the spectrophotometer with an aliquot of QIAsafe matrix. Rehydrate an unused QIAsafe DNA Tube or well of a QIAsafe DNA 96-Well Plate containing only QIAsafe matrix (no DNA) using the same volumes (including dilutions) used for sample preparation.

Appendix B: Quantitative PCR Using Samples Stored in QIAsafe DNA Tubes or 96-Well Plates

Samples recovered from dry storage in QlAsafe matrix can be used directly in real time quantitative PCR without further purification. However, a dye in the QlAsafe matrix can interfere with data acquisition during real-time PCR if the concentration is too high. This dye is present in the QlAsafe matrix with 100 dye equivalents per QlAsafe DNA Tube or per well of a QlAsafe DNA 96-Well Plate.

For real-time PCR using sequence-specific probes or SYBR Green for detection, the dye will not interfere with data acquisition if the final concentration in the PCR is 1 dye equivalent/ μ l or less. For example, if samples were rehydrated in 20 μ l (5 dye equivalents/ μ l), up to 10 μ l can be used in a 50 μ l reaction (50 dye equivalents/50 μ l = 1 dye equivalent/ μ l).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at <u>www.qiagen.com/RefDB/search.asp</u> or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.		
QIAsafe DNA Tubes (50)	50 QIAsafe DNA Tubes in moisture-barrier foil packages	159104		
QIAsafe DNA 96-Well Plates (10)	10 QIAsafe DNA 96-Well Plates in moisture-barrier foil packages, 10 QIAsafe Seals	159112		
Related products				
QIAamp DNA Micro Kit — for purification of total (genomic and mitochondrial) DNA from small amounts of fresh or frozen blood, tissue, and dried blood spots				
QIAamp DNA Micro Kit (50)*	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304		
MinElute PCR Purification Kit — for purification of PCR products (70 bp to 4 kb) in low elution volumes				
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004		
MinElute PCR Purification Kit (250)*	250 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28006		

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at <u>www.qiagen.com</u> or can be requested from QIAGEN Technical Services or your local distributor.

^{*} Fully automatable on the QIAcube®. See <u>www.qiagen.com/MyQIAcube</u> for protocols.

Notes

Notes

Trademarks: QIAGEN®; QIAamp®, QIAcube®, QIAsafe™, MinElute® (QIAGEN Group); Biomatrica®, SampleMatrix® (Biomatrica, Inc.).

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the QIAsafe DNA Tube or QIAsafe 96-Well Plate to the following terms:

- The QIAsafe DNA Tube or QIAsafe DNA 96-Well Plate may be used solely in accordance with the QIAsafe DNA Tube and 96-Well Plate Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the QIAsafe DNA Tube and 96-Well Plate Handbook and additional protocols available at www.giagen.com.
- 2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
- 3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
- 4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
- 5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

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