

June 2015

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# MaXtract<sup>®</sup> High Density Handbook

For improved recovery of nucleic acids during  
organic extraction procedures



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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

<b>MaXtract High Density</b>	<b>(200 x 1.5 ml)</b>	<b>(200 x 2 ml)</b>	<b>(100 x 15 ml)</b>	<b>(25 x 50 ml)</b>
<b>Catalog no.</b>	<b>129046</b>	<b>129056</b>	<b>129065</b>	<b>129073</b>
MaXtract High Density Tubes (yellow)	200 x 1.5 ml	200 x 2 ml	–	–
MaXtract High Density Tubes (clear)	–	–	100 x 15 ml	25 x 50 ml
Quick-Start Protocol	1	1	1	1

## Storage

MaXtract High Density gel should be stored at room temperature (15–25°C) and remains stable for 1 year. It should not be frozen.

## Intended Use

MaXtract High Density is intended for molecular biology applications. This product is 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.

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of MaXtract High Density is tested against predetermined specifications to ensure consistent product quality.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

## Introduction

MaXtract High Density gel enables improved recovery of nucleic acids and ensures ease of handling when working with standard organic extraction mixtures (1). It acts as a barrier between the organic and aqueous phases, allowing the nucleic acid-containing phase to be easily removed by decanting or pipetting. Compared to traditional organic extraction methods, use of MaXtract High Density gel can result in the recovery of 20–30% more nucleic acid. Multiple extractions can be performed in the same tube as long as the maximum sample volume is not exceeded.

## Principle and procedure

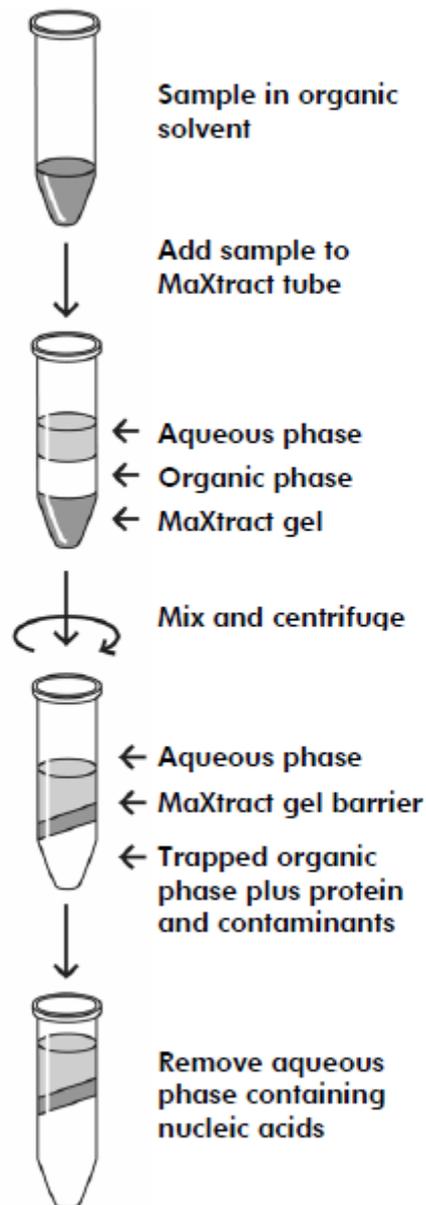
After conventional organic extraction, it is often difficult to recover pure nucleic acid that is free from denatured protein from the aqueous, upper phase. Denatured protein is usually present in the aqueous and organic phase interface. When MaXtract High Density gel is added to phenol:chloroform and chloroform:isoamyl alcohol extractions, application of centrifugal force causes it to migrate below the aqueous upper phase to form a seal between the organic and aqueous phases. The organic phase and the interphase material are trapped below the gel. The gel forms a durable barrier that allows the aqueous upper phase, containing the nucleic acid, to be recovered quantitatively by simply decanting or pipetting to a fresh tube. MaXtract High Density gel allows easy phase separation during nucleic acid isolation procedures that use organic solvents, such as QIAzol<sup>®</sup> Lysis Reagent. The gel forms a stable barrier that traps the organic and intermediate phases at the bottom of the tube so that the aqueous phase can be removed simply by decanting. This leads to improved yield and purity of nucleic acids, as well as enhanced safety and convenience when using organic solvents. The ability of MaXtract High Density gel to separate the phases is based on the density differences between the aqueous and organic media. The organic layer must have a higher density than the MaXtract High Density gel and aqueous phase, and the MaXtract High Density gel must have a higher density than the aqueous phase. High salt and protein concentrations in the aqueous phase have an effect, after mixing with the organic phase, on the densities of both phases. Different organic phase formulations also vary in density.

MaXtract High Density is compatible with RNeasy<sup>®</sup> Kits that involve lysis using QIAzol Lysis Reagent and phase separation, such as RNeasy Plus Universal Tissue Kits, RNeasy Lipid Tissue Kits, and miRNeasy Kits.

## **Compatibility of MaXtract High Density with standard reactions**

MaXtract High Density gel is inert, heat stable, and does not interfere with standard nucleic acid restriction and modification enzymes. Many reactions can be carried out in the presence of MaXtract High Density gel at the appropriate temperature and then terminated by extraction with phenol or phenol:chloroform, or QIAzol Lysis Reagent. MaXtract High Density gel can be present during heat inactivation of enzymes (65°C for 10 minutes) before organic extraction.

## MaXtract Procedure



## 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 safety data sheets (SDSs), available from the product supplier.

### For all protocols

- Microcentrifuge tubes or polypropylene centrifuge tubes (15 ml or 50 ml)
- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Microcentrifuge or centrifuge
- Phenol, saturated with water or Tris buffer (or, for RNA extraction, citric acid or QIAzol Lysis Reagent)
- Chloroform
- Isoamyl alcohol
- Isopropanol
- Ethanol (96–100% and/or 70%)

## Important Notes

### Compatibility of MaXtract High Density

**Table 1. Compatibility of MaXtract High Density with organic and aqueous phases**

Aqueous phase	Organic phase		
	Phenol*	Phenol*:chloroform (1:1)/ phenol*:chloroform: isoamyl alcohol (25:24:1)	Chloroform: isoamyl alcohol (24:1)
<0.5 M NaCl, <1 mg/ml protein	O	H	H
≥0.5 M NaCl	O	H	H
≥1 mg/ml protein	O	H	H
Plasmid DNA isolation <sup>†</sup>	O	H	H
Genomic DNA isolation <sup>‡</sup>	O	H	H
RNA isolation <sup>§</sup>	O	H	H

H: MaXtract High Density; O: This combination of organic and aqueous phases is not suitable for use with MaXtract.

\* Phenol may be saturated with water or with Tris buffer.

<sup>†</sup> Bacterial cleared lysates prepared by alkaline lysis (2).

<sup>‡</sup> Protocols in which nuclei are first pelleted from cells lysed in the presence of 1% Triton<sup>®</sup> X-100, and then lysed in saline/EDTA and SDS, with or without proteinase K (3).

<sup>§</sup> Preparation of total RNA using guanidinium isothiocyanate and organic extraction (4, 5). MaXtract High Density gel is not recommended for use with RNAzol<sup>®</sup> reagent. MaXtract High Density can be used with TRIzol<sup>®</sup> or QIAzol reagents for isolation of total RNA in most cases. Use the volumes recommended in the TRIzol or QIAzol protocol and the centrifuge settings recommended in the MaXtract gel protocol. In certain applications, it may be necessary to add up to another volume of chloroform to achieve optimal phase separation.

## MaXtract applications and protocols

Select the appropriate MaXtract High Density Tube size for your sample volume, as indicated in Table 2.

**Table 2. MaXtract tube sample volumes**

<b>MaXtract High Density Tube</b>	<b>Sample volume</b>	<b>Tube color</b>
1.5 ml, MaXtract High Density	100–500 $\mu$ l	Yellow
2 ml, MaXtract High Density	100–750 $\mu$ l	Yellow
15 ml, MaXtract High Density	1–6 ml	Clear*
50 ml, MaXtract High Density	5–20 ml	Clear*

\* MaXtract High Density is opaque.

## Disruption and homogenizing starting material

Efficient disruption and homogenization of tissue samples can be carried out rapidly and efficiently using either the TissueRuptor<sup>®</sup> (for processing samples individually) or the TissueLyser (for processing multiple samples simultaneously). Disruption and homogenization with the TissueRuptor or TissueLyser generally results in higher RNA yields than with other methods. Additional protocols for more specialized applications are provided in the handbooks supplied with the TissueLyser and TissueRuptor, and in user-developed protocols at [www.qiagen.com](http://www.qiagen.com).

# Protocol: Standard DNA Purification Using MaXtract High Density

The MaXtract High Density procedure can be used for the majority of DNA purification applications and for methods where organic solvents were used. Additional protocols for more specialized applications can be found at [www.qiagen.com/DNA](http://www.qiagen.com/DNA). In addition, QIAGEN provides a wide range of alternative DNA purification kits (that do not require use of phenol/chloroform) for specific purification methods and for different starting materials. Visit [www.qiagen.com/DNA](http://www.qiagen.com/DNA) to find the right kit for your needs.

## Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C).
- All organic solvents should be used at room temperature (15–25°C).
- Do not vortex MaXtract samples or put them into a high-speed shaker for mixing. **It is critical that all mixing steps are performed manually.**
- Use correct centrifuge adapters to ensure tubes are properly supported during centrifugation.
- Do not exceed maximal centrifugal force of 3500 x g for 15 ml and 50 ml MaXtract High Density Tubes.
- Text marked with a ▲ denotes instructions for 1.5 ml and 2 ml MaXtract High Density Tubes; text marked with a ■ denotes instructions for 15 ml and 50 ml MaXtract High Density Tubes.

## Procedure

1. **Immediately before use, pellet MaXtract High Density by centrifugation ▲ at 12,000–16,000 x g for 20–30 s in a microcentrifuge or ■ at 1500 x g for 1–2 min in a standard centrifuge.**
2. **Add the volumes of aqueous sample indicated in the table below and an equal volume of organic extraction solvent directly to the MaXtract High Density Tube prepared in step 1.**

**Table 3. MaXtract High Density Tube sample volumes**

Cat. no.	MaXtract High Density Tube	Sample volume	Tube color
129046	▲ 1.5 ml	100–500 $\mu$ l	Yellow
129056	▲ 2 ml	100–750 $\mu$ l	Yellow
129065	■ 15 ml	1–6 ml	Clear*
129073	■ 50 ml	5–20 ml	Clear*

\* MaXtract High Density is opaque.

**3. Mix the organic and aqueous phases thoroughly to form a transiently homogeneous suspension.**

**IMPORTANT:** Do not vortex.

**4. Centrifuge ▲ at 12,000–16,000 x g for 5 min or ■ at 1500 x g for 5 min to separate the phases.**

MaXtract High Density gel will form a barrier between the aqueous and organic phases. A small amount of the gel may remain in the bottom of the tube. If a second extraction is necessary, and if the maximum tube volume is not exceeded, more organic extraction solvent can be added to the same tube, mixed, and re-centrifuged.

**5. Carefully remove the upper, nucleic acid-containing, aqueous phase by decanting or pipetting into a fresh tube.**

**6. Precipitate the nucleic acid by adding salt, alcohol, and carrier (if required), as recommended for your downstream application.**

See Appendix A (page 22) for information about specific DNA extraction methods — from recovery of DNA from low-melting-point agarose and extraction of phage DNA to purification of plasmid DNA and DNA from FFPE samples, blood, and mouse tails.

## Protocol: Standard RNA Purification Using MaXtract High Density

MaXtract High Density can be used for total RNA extraction from all types of washed and pelleted cultured cells, or for direct extraction of RNA from cultured cells grown in a monolayer, including disruption and homogenization in QIAzol Lysis Reagent. This monophasic solution of phenol and guanidine thiocyanate is designed to facilitate lysis all kinds of tissue (including fatty tissues), and inactivate RNases and sequester DNA away from RNA.

RNA in harvested tissue can be protected against unwanted changes in gene expression by immediately immersing in RNAlater<sup>®</sup> RNA Stabilization Reagent or Allprotect Tissue Reagent at room temperature. For stabilization of DNA, RNA, and protein in adipose and brain tissue samples, we recommend the Allprotect Tissue Reagent. The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in QIAzol Lysis Reagent, samples can be stored at  $-70^{\circ}\text{C}$  for at least 1 month. Additional protocols for more specialized applications are also provided in the respective kit handbooks at [www.qiagen.com](http://www.qiagen.com).

Fast and convenient alternatives that can be used in combination with MaXtract High Density Tubes, are RNeasy Plus Universal Kits, which enable more effective elimination of genomic DNA, and RNeasy Lipid Tissue Kits. These kits combine QIAzol- and phenol/guanidine-based sample lysis with silica-membrane purification of total RNA, and deliver comparable quality and yields using a straightforward procedure utilizing just one product rather than multiple products for precipitation and cleanup steps. RNeasy Lipid Tissue Kits are designed for optimal lysis of tissues rich in fat, such as brain and adipose tissue. The procedure for both kits ensures enrichment for mRNA, since most RNAs that are  $<200$  nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded. RNeasy Plus Universal Kits also enable purification of microRNA (miRNA) and other small RNAs.

### Purification of microRNA

The recommended QIAGEN kits for the purification of total RNA containing small RNAs are miRNeasy Kits, which are also compatible with MaXtract High Density Tubes. They enable purification of total RNA, including RNA that is larger than approximately 18 nucleotides (nt), from all types of animal tissues and cells, including difficult-to-lyse tissues. Alternatively, an miRNA-enriched fraction and a total RNA ( $>200$  nt) fraction can be purified separately (for separate purification, the RNeasy MinElute<sup>®</sup> Cleanup Kit is required). miRNeasy

Kits are available in both spin-column and 96-well formats. For more details, visit [www.qiagen.com/miRNA](http://www.qiagen.com/miRNA).

## Standard RNA extraction using MaXtract High Density

The MaXtract procedure can be used for the majority of RNA extractions. The recommended protocol below can be used with QIAzol Lysis Reagent, as well as similar reagents (e.g., TriReagent, Trizol, etc.). Additionally, QIAGEN also provides a wide range of kits that enable purification of RNA from different samples sources without the need for phenol/chloroform treatment. Visit [www.qiagen.com/RNA](http://www.qiagen.com/RNA) to find the right kit for your needs.

### Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C).
- All organic solvents should be used at room temperature (15–25°C).
- Do not vortex MaXtract samples or put them into a high-speed shaker for mixing. **It is critical that all mixing steps are performed manually.**
- Use correct centrifuge adapters to ensure tubes are properly supported during centrifugation.
- Do not exceed maximal centrifugal force of 3500 x g for 15 ml and 50 ml MaXtract High Density Tubes.
- Text marked with a ▲ denotes instructions for 1.5 ml and 2 ml MaXtract High Density Tubes; text marked with a ■ denotes instructions for 15 ml and 50 ml MaXtract High Density Tubes.

### Procedure

1. **Immediately before use, pellet MaXtract High Density by centrifugation ▲ at 12,000–16,000 x g for 20–30 s in a microcentrifuge or ■ at 1500 x g for 1–2 min in a standard centrifuge.**
2. **Add the volume of QIAzol homogenate (1 ml QIAzol Lysis Reagent per 100 mg tissue) as indicated in Table 4 directly to the MaXtract High Density Tube prepared in step 1.**

**Note:** If the homogenate was derived from tissue stabilized in RNA/ater RNA Stabilization Reagent, add 50–100 µl RNase-free water to the MaXtract High Density Tube as well.

**Table 4. MaXtract High Density Tube sample volumes**

Cat. no.	MaXtract High Density Tube	Sample volume	Tube color
129046	▲ 1.5 ml	100–500 $\mu$ l	Yellow
129056	▲ 2 ml	100–750 $\mu$ l	Yellow
129065	■ 15 ml	1–6 ml	Clear*
129073	■ 50 ml	5–20 ml	Clear*

\* MaXtract High Density is opaque.

- To the MaXtract High Density Tube, add 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent pipetted in step 2. Securely cap the tube, and shake it, mixing the organic and aqueous phases thoroughly for 15 s to form a transiently homogeneous suspension.**

**IMPORTANT:** Do not vortex.

Thorough mixing is important for subsequent phase separation.

- Place the MaXtract High Density Tube on the benchtop at room temperature for 2–3 min.**
- Centrifuge ▲ at 12,000–16,000 x g for 5 min or ■ at 1500 x g for 5 min at 4°C to separate the phases.**

After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase (MaXtract High Density gel); and a lower, red, organic phase containing protein. A small amount of the gel may remain in the bottom of the tube. For tissues with an especially high fat content, an additional clear phase may be visible below the red, organic phase. The volume of the aqueous phase is approximately 60% of the volume of the QIAzol Lysis Reagent pipetted in step 2.

- Transfer the upper, aqueous phase by decanting or pipetting to a fresh tube.**

If a second extraction is necessary, and the maximum tube volume is not exceeded, more organic extraction solvent can be added to the same tube, mixed, and re-centrifuged.

- For precipitation of RNA from the aqueous phase, add 0.5 ml isopropanol per 1 ml QIAzol Lysis Reagent pipetted in the lysis steps before. Mix thoroughly.**
- Place the tube on the benchtop at room temperature for 10 min.**

**9. Centrifuge at 12,000 x g for 10 min at 4°C.**

**10. Carefully aspirate and discard the supernatant.**

The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.

**11. Add at least 1 ml of 75% ethanol per 1 ml QIAzol Lysis Reagent pipetted in the lysis steps before. Centrifuge at 7500 x g for 5 min at 4°C.**

If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x g for 5 min at 4°C.

**12. Remove the supernatant completely, and briefly air-dry the RNA pellet.**

Do not dry the RNA using a vacuum.

**13. Redissolve the RNA in an appropriate volume of RNase-free water. Clean up the RNA using the RNeasy MinElute Cleanup Kit or using the RNeasy Mini, Midi, or Maxi Kits.**

We recommend RNA cleanup to remove contaminating phenol. The presence of residual phenol can result in overestimation of RNA yield and inhibition of enzymatic action in downstream applications. The removal of contaminants by RNA cleanup also improves the stability of the RNA during storage. The RNeasy MinElute Cleanup Kit and RNeasy Mini, Midi, and Maxi Kits allow cleanup of up to 45 µg, 100 µg, 1 mg, and 6 mg total RNA, respectively. For details, refer to the RNA cleanup protocol in the respective kit handbooks supplied at [www.qiagen.com](http://www.qiagen.com).

## 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: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). 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 [www.qiagen.com](http://www.qiagen.com)).

### Comments and suggestions

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#### **MaXtract gel is not forming phases correctly**

- |                                                                                          |                                                                                                           |
|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| a) Incorrect formulation used                                                            | Check the table on page 10 to ensure the choice of MaXtract High Density is correct for your application. |
| b) Incorrect centrifuge speed                                                            | Ensure that the centrifuge speed given in the protocol is used.                                           |
| c) MaXtract High Density gel may have been frozen                                        | Store the MaXtract gel at room temperature (15–25°C).                                                     |
| d) MaXtract High Density tubes were not prepared by centrifugation before the extraction | Prepare the MaXtract High Density tubes by centrifugation before performing the organic extraction.       |

#### **MaXtract High Density migrates to the top of both the aqueous and organic phases**

- |                                           |                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Aqueous layer is denser than the MaXtract | Remove the liquid under the MaXtract High Density gel by piercing the gel with a pipet tip. Use a second pipet tip to recover the liquid and transfer to a MaXtract High Density Tube that has been prepared by centrifugation. Add molecular biology grade water or an appropriate buffer to dilute the sample. Continue as described in the protocol. |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## Comments and suggestions

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### **MaXtract High Density gel remains on the bottom of the tube**

- |                                                                      |                                                                                                  |
|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| a) Incorrect formulation                                             | Check the table on page 10 to ensure that MaXtract High Density is correct for your application. |
| b) Organic phase not sufficiently dense to remain below the MaXtract | Add chloroform to increase the density of the organic phase.                                     |

### **MaXtract High Density gel is forming phases but does not appear uniform**

- |                              |                                                                                                                                                                                                                    |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Phases do not appear uniform | If the barrier is intact, proceed with the protocol. If there is a hole in the barrier, retrieve the sample and place in a fresh MaXtract High Density Tube. Add chloroform and proceed according to the protocol. |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## 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 [www.qiagen.com/RefDB/search.asp](http://www.qiagen.com/RefDB/search.asp) or contact QIAGEN Technical Services or your local distributor.

### Cited references

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## Extraction of total RNA

Chirgwin, J.M. et al. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156.

Birnboim, H.C. (1988) Rapid extraction of high molecular weight RNA from cultured cells and granulocytes for Northern analysis. *Nucleic Acids Res.* **16**, 1487.

## **Appendix A: QIAGEN solutions for specific DNA extraction methods**

### **Recovery of DNA from low melting point agarose**

For gel extraction or cleanup of up to 10  $\mu\text{g}$  DNA, the QIAquick<sup>®</sup> Gel Extraction Kit is recommended. The kit includes spin columns, buffers, and collection tubes for silica-membrane-based purification of DNA fragments from gels (up to 400 mg slices) or enzymatic reactions. DNA from 70 bp to 10 kb is purified using a simple and fast bind-wash-elute procedure in an elution volume of 30–50  $\mu\text{l}$ . An integrated pH indicator allows easy determination of the optimal pH required for DNA binding to the spin column. The procedure can also be fully automated on the QIAcube<sup>®</sup>.

### **Purification of M13/phage DNA**

The efficiency of standard protocols for the isolation of single-stranded DNA (6, 7) may be improved through the use of MaXtract High Density gel (8).

An easy and fast alternative is the QIAprep<sup>®</sup> Spin M13 Kit, which provides silica-based spin columns suitable for processing by vacuum or centrifugation — with no extraction or precipitation steps. In just 30 minutes, the kit yields up to 10  $\mu\text{g}$  of high-purity single-stranded DNA from a 3 ml phage supernatant that is highly suitable for sequencing and site-directed mutagenesis.

### **Purification of $\lambda$ DNA**

MaXtract High Density Tubes can be used in different purification methods for  $\lambda$  DNA (9).

### **Purification of genomic DNA from blood or cultured cells**

The QIAamp<sup>®</sup> DNA Mini Kit simplifies isolation of genomic DNA from human tissue tissues, swabs, CSF, blood, body fluids, or washed cells from urine with fast silica-membrane-based spin-column or vacuum procedures without phenol-chloroform extraction. The spin-column procedure does not require mechanical homogenization, so the total hands-on preparation time is only 20 minutes. Purification of DNA using the QIAamp DNA Mini Kit can be automated on the QIAcube. QIAamp DNA technology yields genomic, mitochondrial, bacterial, parasite or viral DNA from human tissue samples ready to use in PCR and blotting procedures.

## **Purification of genomic DNA from mouse tails**

MaXtract High Density can be used for the purification of genomic DNA from mouse tails containing organic solvent left over from the purification protocol. Because genomic DNA is fragile and is easily sheared by mechanical forces, it is important to avoid vortexing. For this reason, it is recommended to use large-bore pipet tips when handling genomic DNA in solution.

As an easy alternative, QIAGEN offers DNeasy<sup>®</sup> Blood & Tissue Kits which provide fast and easy silica-based DNA purification from a wide range of sample types, including animal species commonly encountered in life science, veterinary, and genotyping applications in convenient spin-column and 96-well-plate formats. Most samples can be directly lysed with proteinase K, eliminating the need for mechanical disruption and reducing hands-on time. Optimized protocols for specific sample types provide reproducible purification of high-quality DNA for life science, genotyping, and veterinary pathogen research applications. Purification of DNA using the DNeasy Blood & Tissue Kits can be automated on the QIAcube.

## **Purification of plasmid DNA**

MaXtract High Density can be used in small- and large-scale protocols based on an alkaline lysis procedure (1, 2, 10). The basic protocol works with *E. coli* cultures grown for 12 to 14 hours in up to 500 ml of LB or DYT, or in 250 ml Terrific Broth (see page 24).

For isolation of up to 20  $\mu\text{g}$  high-purity molecular biology grade plasmid or cosmid DNA, the QIAprep Spin MiniPrep Kit is recommended. This kit is designed for use in routine molecular biology applications such as fluorescent and radioactive sequencing and cloning. QIAprep membrane technology eliminates time-consuming phenol-chloroform extraction and alcohol precipitation, as well as the problems and inconvenience associated with loose resins and slurries. High-purity plasmid DNA eluted from QIAprep spin columns is immediately ready to use and there is no need to precipitate, concentrate, or desalt.

## **Purification of DNA from FFPE samples**

The QIAamp DNA FFPE Tissue Kit is specially designed for purifying DNA from formalin-fixed, paraffin-embedded tissue sections. The kit uses special lysis conditions to release DNA from tissue sections and to overcome inhibitory effects caused by formalin crosslinking of nucleic acids. QIAamp MinElute spin columns are optimized for purification of high-quality DNA in small volumes. Purification of DNA using the QIAamp DNA FFPE Tissue Kit can be automated on the QIAcube.

## Appendix B: Composition of Buffers

Buffer compositions are given per liter of solution.

### TE buffer

Dissolve 1.21 g Tris base and 0.37 g  $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$  in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

### Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml distilled water. Adjust the pH to 7.0 with 1 M NaOH. Adjust the volume to 1 liter with distilled water. Sterilize by autoclaving.

### Preparation of LB- $\text{MgSO}_4$ medium

Prepare LB medium and add  $\text{MgSO}_4$  to a final concentration of 1 mM. Sterilize by autoclaving.

### Preparation of DYT medium

Dissolve 10 g tryptone, 10 g yeast extract, and 2 g glucose in 1 liter distilled water. Sterilize by autoclaving.

### Preparation of Terrific Broth

Dissolve 12 g tryptone, 24 g yeast extract, 4 ml glycerol, 2.31 g  $\text{KH}_2\text{PO}_4$ , and 12.54 g  $\text{K}_2\text{HPO}_4$  in 1 liter distilled water. Sterilize by autoclaving.

## Ordering Information

Product	Contents	Cat. no.
MaXtract High Density (200 x 1.5 ml)	200 x 1.5 ml MaXtract High Density Tubes	129046
MaXtract High Density (200 x 2 ml)	200 x 2 ml MaXtract High Density Tubes	129056
MaXtract High Density (100 x 15 ml)	100 x 15 ml MaXtract High Density Tubes	129065
MaXtract High Density (25 x 50 ml)	25 x 50 ml MaXtract High Density Tubes	129073
<b>Related products</b>		
<b>RNeasy Plus Universal Kits — for purification of total RNA from all types of tissue using gDNA Eliminator Solution</b>		
RNeasy Plus Universal Mini Kit (50)*	For 50 RNA minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Solution, Collection Tubes, RNase-Free Water and Buffers	73404
<b>RNeasy Lipid Tissue Mini Kit — for purification of up to 100 µg total RNA from fatty tissues and other types of tissue</b>		
RNeasy Lipid Tissue Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-free Reagents and Buffers	74804
<b>miRNeasy Mini Kit — For purification of microRNA and total RNA from tissues and cells</b>		
miRNeasy Mini Kit (50)*	For 50 preps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzol Lysis Reagent, RNase-Free Reagents and Buffers	217004

\* Other kit sizes and/or formats available; see [www.qiagen.com](http://www.qiagen.com).

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
<b>TissueLyser LT — for low- to medium-throughput sample disruption for molecular analysis</b>		
TissueLyser LT	Compact bead mill, 100-240 V AC, 50-60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)*	85600
<b>TissueLyser II — for medium- to high-throughput sample disruption for molecular analysis</b>		
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)†	85300
<b>TissueRuptor — for low-throughput sample disruption for molecular analysis</b>		
TissueRuptor (120 V, 60 Hz, US/JP)	Handheld rotor–stator homogenizer, 120 V, 60 Hz (for North America and Japan), 5 TissueRuptor Disposable Probes	9001271
TissueRuptor (230 V, 50/60 Hz, EU/CH)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for Europe [excluding UK and Ireland]), 5 TissueRuptor Disposable Probes	9001272
TissueRuptor (230 V, 50/60 Hz, UK)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for UK and Ireland), 5 TissueRuptor Disposable Probes	9001273
TissueRuptor (230 V, 50/60 Hz, AUS)	Handheld rotor–stator homogenizer, 230 V, 50/60 Hz (for Australia), 5 TissueRuptor Disposable Probes	9001274

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 [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

\* The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

† The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

## Notes



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