



illustra

GFX PCR DNA and Gel
Band Purification Kit

Product booklet

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1 Introduction

Product codes

28903470 (100 purifications)

28903471 (250 purifications)

About

For the purification and concentration of DNA from PCR mixtures, restriction enzyme digestions, solutions and agarose gel bands.

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

It is the responsibility of the user to verify the use of the illustra™ GFX™ PCR DNA and Gel Band Purification Kit for a specific application as the performance characteristics of this kit have not been verified for any specific organism.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

**NOTICE**

This protocol requires the use of ethanol.

The chaotrope in Capture buffer type 2 is harmful if ingested, inhaled, or absorbed through the skin, and can cause nervous system disturbances, severe irritation, and burning. High concentrations are extremely destructive to the eyes, skin, and mucous membranes of the upper respiratory tract. Gloves should always be worn when handling this solution.

**CAUTION**

If contact with skin or eyes should occur, wash immediately with water.

Storage

Store at room temperature (20–25°C)

Expiry

For expiry date please refer to outer packaging label.

2 Components

Kit contents

Identification	PackSize	10 purifications	100 purifications	250 purifications
	Product code	Sample Pack	28903470	28903471
Blue	Capture ¹ buffer type 3	10 ml	55 ml	140 ml
Yellow	Wash buffer type 1	2.5 ml (Add 10 ml absolute ethanol before use)	25 ml (Add 100 ml absolute ethanol before use)	62.5 ml (Add 250 ml absolute ethanol before use)
Grey	Elution ² buffer type 4	12 ml	12 ml	28 ml
Pink	Elution ³ buffer type 6	12 ml	12 ml	28 ml
	illustra GFX MicroSpin™ Columns	10	2 × 50	5 × 50
	Collection tubes	10	2 × 50	5 × 50

¹ Capture buffer type 3 contains a pH indicator that changes color at various pH levels to identify whether the Capture buffer plus sample mix is at the optimal pH for DNA to bind to the silica membrane. Note that sufficient Capture buffer type 3 is provided to purify 100/250 gel bands (depending on pack size) weighing up to 500 mg. If gel bands larger than 500 mg are purified, there will not be enough Capture buffer type 3 to perform 100/250 purifications.

² Elution buffer type 4 (10 mM Tris-HCl, pH 8.0) can be used for samples for multiple downstream applications and should be used for samples requiring long term storage.

³ Elution buffer type 6 (sterile nuclease free water) should be used for samples to be sequenced only, especially if using a capillary loading analyzer.

Refer to the Certificate of Analysis for a complete list of kit components.

Cytiva supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in other illustra kits e.g. the Capture buffer supplied in the illustra GFX PCR DNA and Gel Band Purification Kit is not the same as the Capture buffer supplied in the illustra CyScribe GFX Purification Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1–14, Wash buffer type 1–6 and Elution buffer type 1–8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

Note that the GFX MicroSpin columns and Collection tubes have a frosted area on the surface for writing on and easy labeling of samples. Also note that the cap of the Collection tube will fit onto the GFX MicroSpin column when it is inserted into the Collection tube.

Materials to be supplied by user

Disposables:

1.5 ml DNase-free microcentrifuge tubes (2 per purification from agarose gels, 1 per purification from PCR mixtures and enzyme reactions)

Chemicals:

Absolute ethanol

Equipment needed

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes
Vortex mixer

For purification of DNA from agarose gels only:

- Clean scalpel or razor blade
- Water bath or heat-block for 60°C incubation

3 Description

Background

The illustra GFX PCR DNA and Gel Band Purification Kit is designed for the purification and concentration of DNA from PCR mixtures, restriction enzyme digestions, solutions and agarose gel bands. DNA ranging in size from 50 bp up to 10 kbp can be purified from solution volumes of up to 100 μ l and from gel slices of up to 900 mg. No modifications are required for purification of DNA from gels run in borate based buffers (e.g. TBE).

The Capture buffer type 3 contains a pH indicator that changes color at various pH levels to visually indicate whether the Capture buffer plus sample mix is at the optimal pH for DNA to bind to the silica membrane.

For efficient binding of DNA to the silica membrane the Capture buffer-sample mix requires a $\text{pH} \leq 7.5$. The pH indicator will appear a yellow or pale orange color in this range. If the pH is > 7.5 (which can occur if the agarose gel electrophoresis buffer is not refreshed, is incorrectly prepared, or if the pH of the sample exceeds the buffering capacity of Capture buffer) DNA adsorption will be inefficient and yield may be reduced. The pH indicator will appear a dark pink or red color in this range. If the pH of the binding mixture is > 7.5 it can be adjusted by addition of a small volume of 3 M sodium acetate pH 5.0 before loading onto the GFX MicroSpin column.

The indicator dye does not interfere with DNA binding and is completely removed during the wash step. In addition, using a colored as opposed to a clear binding mixture allows easy visualization of any unsolubilized agarose. Complete solubilization is necessary to obtain maximum yields.

If pH indicator is a yellow or pale orange color, Capture buffer sample mix is at optimal pH for efficient DNA binding to the silica membrane.

If the pH indicator is a dark pink or red color, the pH of the Capture buffer-sample mix is too high to achieve efficient DNA adsorption to the silica membrane.

Typical reactions from which DNA can be isolated include:

- Sequential restriction enzyme digests, where the enzymes involved have differing buffer requirements; perform the first digest, purify the sample using the illustra GFX PCR DNA and Gel Band Purification Kit, and proceed to the second enzyme reaction.
- DNA modified by an enzymatic reaction, including phosphatase reactions with CIP or SAP, filling-in or removal of overhangs to form blunt ends e.g. with DNA (Klenow) polymerase I, large fragment or T4 DNA polymerase or proof reading polymerases, and nuclease reactions e.g. S1 nuclease or mung bean nuclease.

To concentrate your DNA sample, use an elution volume that is less than the starting volume of the sample being purified. For optimal recovery, use 50 μ l elution volume. [Table 1, on page 9](#) shows example percentage yields obtained when purifying a 910 bp fragment.

Table 1. Percentage yield obtained with illustra GFXProduct PCR DNA and Gel Band Purification Kit

Sample Source	Elution volume (μ l)	Yield (%) ¹
PCR	10	65
	50	82
300 mg agarose	10	57
	50	91
900 mg agarose	50	55

¹ 910 bp PCR fragment at 8.4 ng/ μ l was purified from the PCR mixture or from the weight of agarose indicated. Percentage yield was determined by A_{260} readings.

Fragments ranging in size from 48 kbp to 50 bp can be purified. The Figure below shows two DNA ladders purified using the illustra GFX PCR DNA and Gel Band Purification Kit; the fragment sizes are indicated. The standard protocol was followed.

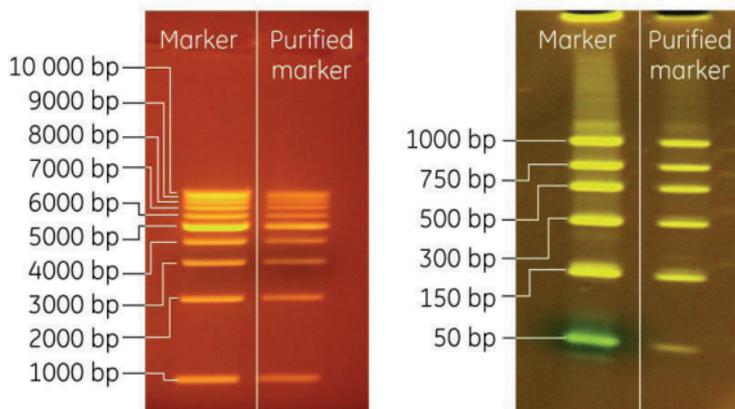


Fig 1. Agarose gel analysis of markers purified using the illustra GFXProduct PCR DNA and Gel Band Purification Kit

Post purified markers were diluted 1 in 3 compared to starting material.

The Table below shows typical yield for fragments of differing sizes.

Table 2. Percentage yield obtained for fragments of differing sizes

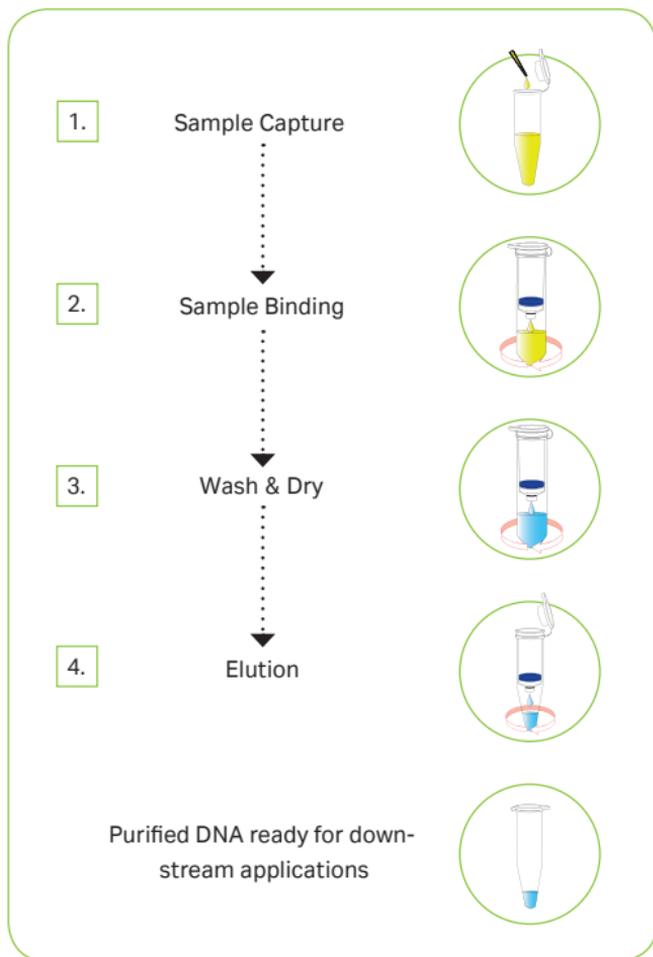
Fragment size	Percent recovery
250	83.7
500	84.7
1 000	94.7
1 500	91.8
2 000	91.1
3 000	95.4
4 000	86.4
6 000	68.1
10 000	42.6

10 μ l of KiloBase DNA Marker was purified using the illustra GFX PCR DNA and Gel Band Purification Kit, and eluted with 50 μ l of sterile DNase-free water. 10 μ l of purified sample was loaded onto a 0.8% agarose gel. For comparison, unpurified marker was diluted 1:5 and 10 μ l loaded onto the gel. ImageQuant™ software was used to determine percent recovery.

The developed method uses a chaotropic agent to extract DNA from solution and/or to dissolve agarose and to denature proteins (1, 2). DNA binds selectively to the silica membrane contained in the illustra GFX MicroSpin column. The matrix-bound DNA is washed with an ethanolic buffer to remove salts and other contaminants, and the purified DNA is eluted in a low ionic strength buffer.

The basic principle

Use of the illustra GFX PCR DNA and Gel Band Purification Kit involves the following steps:



Step	Comments	Component
1. Sample Capture	Capture buffer type 3 is added to the sample. Proteins are denatured and/or agarose is dissolved.	Capture buffer type 3 Blue
2. Sample Binding	The Capture buffer type 3 - sample mix is applied to the illustra GFX MicroSpin Column; DNA binds to the membrane.	illustra GFX MicroSpin Columns
3. Wash & Dry	A combined washing/drying step removes salts and other contaminants from the membrane bound DNA.	Wash buffer type 1 Yellow
4. Elution	Purified sample is eluted from the column with buffer chosen by user. Use Elution buffer type 4 (10 mM Tris-HCl, pH 8.0) for multiple downstream applications and long term storage of samples. Use Elution buffer type 6 (sterile nuclease free water) for samples to be sequenced only.	Elution buffer type 4 Grey OR Elution buffer type 6 Pink

Product specifications

Sample Type:	PCR mixtures, enzyme reactions, DNA solutions, agarose gel slices
Sample size range	48 kbp–50 bp
Input volume	100 µl solution or up to 900 mg agarose
Elution volume	10–50 µl (into one of two elution buffers provided)
Major subsequent applications	Further PCR amplification, sequencing, labeling, restriction enzyme digestion, ligation, cloning.
Yield obtained when purifying a 910 bp fragment at a starting concentration of 8.4 ng/µl	PCR 10 µl elution volume–65% PCR 50 µl elution volume–82% 300 mg agarose 10 µl elution volume–57% 300 mg agarose 50 µl elution volume–91%

When purifying a PCR fragment less than 50 bp (but greater than 10 bases) in length, use an illustra MicroSpin G-25 Column.

This kit cannot be used for the purification of RNA.

If handling large numbers of samples in solution, 100 bp–10 kb in length, use an illustra GFX PCR Purification Kit.

Typical output

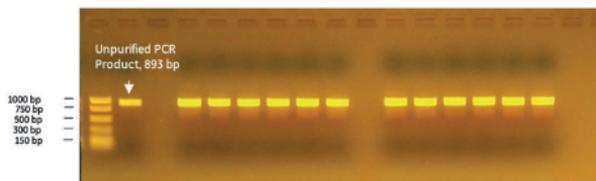


Fig 2. Purified PCR product

Purified p53 PCR fragments (893 bp), ~ 100 ng samples run on a 1% (w/v) agarose gel.

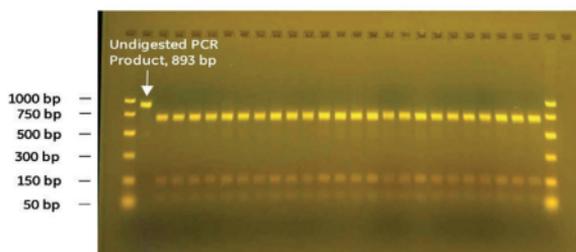


Fig 3. PCR product digested using MscI restriction enzyme

Purified p53 PCR fragments (893 bp) digested with the salt sensitive restriction enzyme MscI (200 ng plasmid DNA, 1 unit MscI, 37°C for 1 hour). Expected fragments from MscI digestion of p53 PCR fragment: 685 bp, 139 bp & 69 bp.

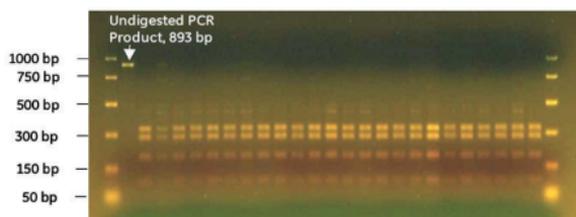


Fig 4. PCR product digested using HpaII restriction enzyme

Purified p53 PCR fragments (893 bp) digested with the salt sensitive restriction enzyme HpaII (200 ng plasmid DNA, 1 unit HpaII, 37°C for 1 hour). Expected fragments from HpaII digestion of p53 PCR fragment: 321 bp, 283 bp, 187 bp & 102 bp.

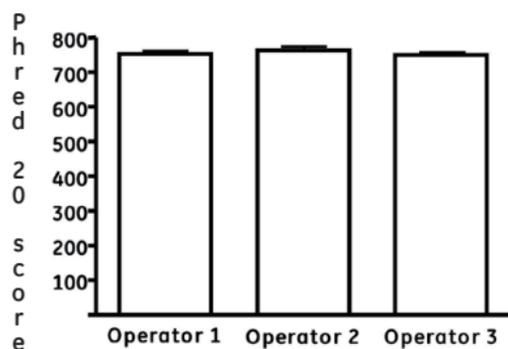


Fig 5. Phred 20 scores as an indication of PCR DNA quality

DNA sequencing	Phred 20 read length
Operator	illustra
1	752 ± 26
2	763 ± 31
3	749 ± 24

p53 PCR fragments (893 bp) were purified by 3 operators and eluted in 10 µl Tris-HCl buffer pH 8.0. All purified PCR fragments were subjected to DNA sequencing and the Phred 20 read length determined for each reaction (n = 6 for each operator).

4 Protocol



NOTICE

Solutions are NOT transferable between Cytiva illustra kits e.g. the composition of the Capture buffer in the GFX PCR DNA and Gel Band Purification Kit is not the same as the Capture buffer in the CyScribe GFX Purification Kit. Please note type number for differentiation.

Use of icons



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Tip: *A tip contains useful information that can improve or optimize your procedures.*

Preparation of working solutions

For Materials & Equipment to be supplied by user see [Materials to be supplied by user, on page 7](#) and [Equipment needed, on page 7](#).

Capture buffer type 3 (Blue)

Capture buffer type 3 contains a pH indicator that changes color at various pH levels to visually indicate whether the Capture buffer plus sample mix is at the optimal pH for DNA to bind to the silica membrane. Refer to [Background, on page 7](#) for more information.

Wash buffer type 1 (Yellow)

Prior to first use, add absolute ethanol to the bottle containing Wash buffer type 1. Add 100 ml of absolute ethanol to the Wash buffer type 1 in kit 28903470 or add 250 ml ethanol to the Wash buffer type 1 in kit 28903471. For the sample pack size, add 10 ml absolute ethanol to the Wash buffer type 1. Mix by inversion. Indicate on the label that this step has been completed. Store upright in an airtight container.

Elution buffers type 4 and 6

Two elution buffers are provided with this kit to enable optimal performance of the purified sample in as wide a range of downstream applications as possible.

Elution buffer type 4 (Grey) (10 mM Tris-HCl, pH 8.0) - the sample should be eluted into this buffer for use in a range of downstream applications and for long term storage of the sample.

Elution buffer type 6 (Pink) (sterile nuclease free water) -the sample should be eluted into this buffer for the best results with sequencing applications, especially when using a salt-sensitive capillary loading analyzer.

Sample verification

When purifying DNA from either PCR mixtures or restriction enzyme digestions, we recommend running an analytical gel prior to purification to check for a single band representing the DNA species of interest. If multiple bands are present, we recommend performing a preparative gel and excising the band of interest and following [Protocol for purification of DNA from TAE and TBE agarose gels, on page 23](#).

Protocol for purification of DNA from solution or an enzymatic reaction

Sample Capture

Step	Action
------	--------

- | | |
|---|---|
| 1 | Add 500 μ l Capture buffer type 3 (blue) to up to 100 μ l sample. |
|---|---|

Tip:

If sample volume is greater than 100 μ l, divide the sample and purify using more than one GFX MicroSpin column.

- | | |
|---|-----------------|
| 2 | Mix thoroughly. |
|---|-----------------|



NOTICE

If sample contains DNA greater than 5 kbp, do not vortex, as this may cause shearing of the DNA.

Step Action

- 3 Check that the Capture buffer type 3-sample mix is yellow or pale orange in color.



NOTICE

If the color of the binding mixture is dark pink or red, add a small volume (~ 10 μ l) of 3 M sodium acetate pH 5.0 and mix. Ensure that the binding mixture turns a yellow or pale orange color before loading onto the GFX MicroSpin column. Refer to [Background, on page 7](#) for more information.

- 4 For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.
 - 5 Proceed with the next part of the protocol
-

Sample Binding

Step Action

- 1 Centrifuge Capture buffer type 3-sample mix briefly to collect the liquid at the bottom of the tube.

Step Action

- 2 Load the Capture buffer type 3-sample mix onto the assembled GFX MicroSpin column and Collection tube.

Tip:

the cap of the Collection tube can be used to cap the GFXMicroSpin column. If the cap is not required cut it off.

- 3 Spin the assembled column and Collection tube at 16 000 × g for 30 seconds.
- 4 Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube.
- 5 Proceed with the next part of the protocol
-

Wash & Dry**Step Action**

- 1 Add 500 µl Wash buffer type 1 (Yellow) to the GFX MicroSpin column.

Step	Action
-------------	---------------

- | | |
|----------|---|
| 2 | Spin the assembled column and Collection tube at 16 000 × g for 30 seconds. |
|----------|---|

Tip:

If purity is paramount (e.g., if the sample is to be used in a blunt-ended ligation), repeat Wash & Dry step a and perform step b twice. After the first spin, discard flow through, place the GFX MicroSpin column back inside the Collection tube and centrifuge at 16 000 × g for an additional 30 seconds. This extra wash step may reduce yield by 4%

- | | |
|----------|---|
| 3 | Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 ml microcentrifuge tube (supplied by user). |
| 4 | Proceed with the next part of the protocol |
-

Elution

Step	Action
-------------	---------------

- | | |
|----------|--|
| 1 | Add 10–50 µl Elution buffer type 4 (Grey) OR type 6 (Pink) to the center of the membrane in the assembled GFX MicroSpin column and sample Collection tube. |
| 2 | Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute. |
| 3 | Spin the assembled column and sample Collection tube at 16 000 × g for 1 minute to recover the purified DNA. |

Step	Action
-------------	---------------

- | | |
|----------|---|
| 4 | Proceed to downstream application. Store the purified DNA at -20°C. |
|----------|---|
-

Protocol for purification of DNA from TAE and TBE agarose gels

Sample Capture

Step	Action
-------------	---------------

- | | |
|----------|---|
| 1 | Weigh a DNase-free 1.5 ml microcentrifuge tube and record the weight. |
| 2 | Using a clean scalpel, long wavelength (365 nm) ultraviolet light and minimal exposure time, cut out an agarose band containing the sample of interest. Place agarose gel band into a DNase-free 1.5 ml microcentrifuge tube (user supplied). |
| 3 | Weigh the microcentrifuge tube plus agarose band and calculate the weight of the agarose slice. |

Tip:

The sample may now be stored at -20°C for up to 1 week.

Step Action

- 4 Add 10 μ l Capture buffer type 3 (Blue) for each 10 mg of gel slice, for example, add 300 μ l Capture buffer type 3 to each 300 mg gel slice



NOTICE

If the gel slice weighs less than 300 mg, add 300 μ l Capture buffer type 3. DO NOT add less than 300 μ l Capture buffer type 3 per sample.

Tip:

To save time when purifying multiple samples of gel bands (each weighing less than 500 mg), add 500 μ l Capture buffer type 3 to each gel slice. DNA recovery will be unaffected providing the volume of Capture buffer type 3 is in excess of the weight of each gel slice.

- 5 Mix by inversion and incubate at 60°C for 15–30 minutes until the agarose is completely dissolved. Mix by inversion every 3 minutes.



NOTICE

If sample contains DNA greater than 5 kb, do not vortex, as this may cause shearing of the DNA.

Step Action

- 6 Once the agarose has completely dissolved check that the Capture buffer type 3-sample mix is yellow or pale orange in color.



NOTICE

If the color of the binding mixture is dark pink or red, add a small volume (~ 10 μ l) of 3 M sodium acetate pH 5.0 and mix. Ensure that the binding mixture turns a yellow or pale orange color before loading onto the GFX MicroSpin column. Refer to [Background, on page 7](#) for more information.

- 7 For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.
- 8 Proceed with the next part of the protocol
-

Sample Binding

Step Action

- 1 Centrifuge Capture buffer type 3- sample mix briefly to collect the liquid at the bottom of the tube.

Step	Action
-------------	---------------

- | | |
|----------|--|
| 2 | Transfer up to 800 µl Capture buffer type 3- sample mix onto the assembled GFX MicroSpin column and Collection tube. |
|----------|--|

Tip:

the cap of the Collection tube can be used to cap the GFX MicroSpin column. If the cap is not required cut it off.

- | | |
|----------|---|
| 3 | Incubate at room temperature for 1 minute. |
| 4 | Spin the assembled column and Collection tube at 16 000 × g for 30 seconds. |
| 5 | Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube. |
| 6 | Repeat Sample Binding steps b. to e. as necessary until all sample is loaded. |
| 7 | Proceed with the next part of the protocol |
-

Wash & Dry

Step	Action
-------------	---------------

- | | |
|----------|---|
| 1 | Add 500 µl Wash buffer type 1 (Yellow) to the GFX MicroSpin column. |
|----------|---|

Step	Action
-------------	---------------

- | | |
|----------|--|
| 2 | Spin the assembled column and Collection tube at 16 000 × g for 30 seconds |
|----------|--|

Tip:

If purity is paramount (e.g., if the sample is to be used in a blunt-ended ligation), repeat Wash & Dry step a and perform step b twice. After the first spin, discard flow through, place the GFX MicroSpin column back inside the Collection tube and centrifuge at 16 000 × g for an additional 30 seconds. This extra wash step may reduce yield by 4%.

- | | |
|----------|---|
| 3 | Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 ml microcentrifuge tube (supplied by user). |
| 4 | Proceed with the next part of the protocol |
-

Elution

Step	Action
-------------	---------------

- | | |
|----------|---|
| 1 | Add 10–50 µl Elution buffer type 4 (grey) <i>OR</i> type 6 (pink) to the center of the membrane in the assembled GFX MicroSpin column and sample Collection tube. |
| 2 | Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute. |
| 3 | Spin the assembled column and sample Collection tube at 16 000 × g for 1 minute to recover the purified DNA. |

Step	Action
------	--------

- | | |
|---|---|
| 4 | Proceed to downstream application. Store the purified DNA at -20°C. |
|---|---|

5 Appendix

RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

$$\text{RPM} = 1000 \times \sqrt{(\text{RCF}/1.12r)}$$

Where RCF = relative centrifugal force, r = radius in mm measured from the centre of the spindle to the bottom of the rotor bucket, and RPM = revolutions per minute.

e.g. If an RCF of $735 \times g$ is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

Troubleshooting guide

This guide may be helpful in the first instance. However, if problems persist, or for further information, please contact Cytiva technical services. Visit [cytiva.com](https://www.cytiva.com) for contact information. Alternatively, log onto [cytiva.com/illustra](https://www.cytiva.com/illustra)

Table 3. Problem: DNA yield is low

Possible cause	Suggestions
<i>Incorrect volume of Capture buffer type 3 used</i>	<ul style="list-style-type: none">• Check ratio of sample to Capture buffer type 3. If sample is a solution, add 500 µl Capture buffer type 3 to a maximum sample volume of 100 µl.

Possible cause	Suggestions
	<ul style="list-style-type: none"> • If sample is an agarose gel slice, add 10 μl Capture buffer type 3 for each 10 mg of gel slice to a maximum sample weight of 900 mg. For maximum yield, divide sample into 300 mg aliquots and perform 3 separate purifications.
<i>Wash buffer type 1 was not completely removed before Elution step</i>	<ul style="list-style-type: none"> • The 30 second centrifugation at 16 000 \times g during the Wash & Dry step is essential for good sample elution. If humidity is high, the centrifugation can be increased to 1 minute at 16 000 \times g.
<i>Agarose gel band was not fully dissolved in the Capture buffer type 3</i>	<ul style="list-style-type: none"> • Visually inspect the agarose-Capture buffer type 3 mix to ensure the agarose has fully dissolved before proceeding. It may take 15 minutes for the agarose gel band to dissolve.
<i>Capture buffer type 3-sample mix was at pH > 7.5</i>	<ul style="list-style-type: none"> • Check that the color of the Capture buffer type 3-sample mix is a yellow or pale orange color before loading onto the GFX MicroSpin column. If the color of the binding mixture is dark pink or red, add a small volume of 3 M sodium acetate pH 5.0 and mix. Continue this until the binding mixture turns yellow or pale orange.

Table 4. Problem: DNA sample floats out of well when loading a gel

Possible cause	Suggestions
<i>Wash buffer type 1 was not completely removed before Elution step</i>	<ul style="list-style-type: none"> • The 30 second centrifugation at 16 000 \times g during the Wash & Dry step is essential for good sample elution. If humidity is high, the centrifugation can be increased to 1 minute at 16 000 \times g.
<i>Collection tube was not emptied after the centrifugation for the Sample Binding step. This can cause the Collection tube to overflow and the Wash & Dry step to be affected.</i>	<ul style="list-style-type: none"> • Always empty the Collection tube after the centrifugation within the Sample Binding step.

Table 5. Problem: DNA appears degraded on gels, or as two bands where previously there was one

Possible cause	Suggestions
<i>Incorrect Elution buffer used</i>	• Use Elution buffer type 4 for fragments that are to be used in cloning applications and for long term storage. Use Elution buffer type 6 to elute fragments that are to be sequenced only.
<i>Sample was not present as a single band prior to purification</i>	• Check the PCR or restriction enzyme digestion gave a single band on an agarose gel prior to purification. If multiple bands are present, isolate the band of interest using a preparative gel and follow Protocol for purification of DNA from TAE and TBE agarose gels, on page 23 .

Table 6. Problem: DNA fails to ligate

Possible cause	Suggestions
<i>Salt or other contaminants present in final purified sample</i>	• Perform a second optional Wash & Dry step as detailed in protocol.

Related products

A full range of Molecular Biology reagents can be found on the Cytiva web site and in the catalog.

cytiva.com/illustra

If you need further information, Cytiva technical services are happy to assist. Visit cytiva.com for contact information.

Application	Product	Product code	Pack size
Purification of DNA from PCR and enzymes; multiple samples	illustra GFX 96 PCR Purification Kit	28903445	10 × 96 well plates

Application	Product	Product code	Pack size
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions
Purification of oligonucleotides and 10–50 bp DNA fragments	illustra MicroSpin G-25 Columns (100–150 µl sample volume)	27532501	50 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50 Columns	27534001	50 purifications
Preparation of circular DNA templates	illustra TempliPhi™ 100 Amplification Kit	25640010	100 reactions
Genomic DNA amplification	illustra GenomiPhi™ V2 DNA Amplification Kit	25660030	25 reactions
	illustra GenomiPhi HY DNA Amplification Kit	25660022	25 reactions
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25150001	100 reactions
	illustra PuReTaq Ready-To-Go™ PCR Beads	27955701	96 reactions in 0.2 ml tubes/plate
	illustra PuReTaq Ready-To-Go PCR Beads	27955702	5 × 96 reactions in 0.2 ml tubes/plate

Application	Product	Product code	Pack size
	FideliTaq™ PCR Master Mix Plus (2 ×)	E71182	100 reactions
	FideliTaq Master Mix Plus	E71183	100 reactions
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix dNTPSet (A,C,G,T) 20 mM each	28406557	10 µmol
	illustra DNA Polymerization Mix dNTPSet (A,C,G,T) 20 mM each	28406558	40 µmol (4 × 10 µmol)
	illustra PCR Nucleotide Mix dNTPSet (A,C,G,T) 25 mM each	28406560	500 µl
	illustra PCR Nucleotide Mix dNTPSet (A,C,G,T) 2 mM each	28406562	1 ml

6 Quick Reference Protocol Card

illustra GFX PCR DNA and Gel Band Purification Kit

A. Protocol for purification of DNA from PCR mixtures or an enzymatic reaction

- Check appropriate volume of ethanol added to Wash buffer type 1.

 :Add  :Spin  :Incubate

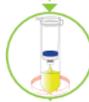
1. Sample capture

-   500 μ l Capture buffer type 3 to up to 100 μ l sample
- Mix thoroughly
- Check color of Capture buffer type 3-sample mix is yellow or pale orange



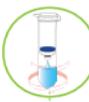
2. Sample binding

-  Capture buffer type 3-sample mix to assembled GFX MicroSpin™ column and Collection tube
-  30 s 16 000 \times g. Discard flow through
- Place GFX MicroSpin column inside the same Collection tube



3. Wash & dry

-   500 μ l Wash buffer type 1
-  30 s 16 000 \times g
- Discard Collection tube. Transfer GFX MicroSpin column to



4. Elution

-   OR  10–50 μ l Elution buffer type 4 OR type 6
-  60 s room temperature
-  60 s 16 000 \times g
- Retain flow through
- Store purified sample DNA at -20°C



B. Protocol for purification of DNA from TAE and TBE agarose gels

- Check appropriate volume of ethanol added to Wash buffer type 1.

 :Add  :Spin  :Incubate

1. Sample capture

- Weigh a DNase-free 1.5 ml microcentrifuge tube
- Excise band of interest and place in microcentrifuge tube
- Weigh microcentrifuge tube plus agarose gel band
- Calculate weight of agarose gel slice
-  10 μ l Capture buffer type 3 for each 10 mg agarose gel slice
- Mix by inversion
-  60°C until agarose is completely dissolved
- Check color of Capture buffer type 3-sample mix is yellow or pale orange



2. Sample binding

-  600 μ l Capture buffer type 3-sample mix to assembled GFX MicroSpin™ column and Collection tube
-  60 s room temperature
- 30 s 16 000 \times g. Discard flow through
- Place GFX MicroSpin column inside the same Collection tube
- Repeat Sample Binding step until all sample is loaded



3. Wash & dry

-  500 μ l Wash buffer type 1
-  30 s 16 000 \times g
- Discard Collection tube. Transfer GFX MicroSpin column to a clean 1.5ml DNase-free microcentrifuge tube.



4. Elution

-  OR  10–50 μ l Elution buffer type 4 OR type 6
-  60 s room temperature
-  60 s 16 000 \times g
- Retain flow through
- Store purified sample DNA at -20°C



7 References

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2. Marko, M. A., Chipperfield, R. & Birnbom, H. C. Anal. Biochem. 121, 382 (1982).



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