illustra
GFX PCR DNA and Gel Band Purification Kit

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

Product booklet

Codes: 28-9034-70 (100 purifications)
28-9034-71 (250 purifications)

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Quick Reference Protocol Card Back Cover
Tear off sheet containing protocols for the experienced user purifying or concentrating DNA from PCR mixtures, restriction enzyme digestions, solutions or agarose gel bands

1. Legal

Product use restriction

The components of the **illustra™ GFX™ PCR DNA and Gel Band Purification Kit** have been designed, developed and sold **for research purposes only**. They are suitable **for *in vitro* use only**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

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.

http://www.gelifesciences.com

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water. (See Material Safety Data Sheet(s) and/or Safety Statements(s) for specific recommendations).

Warning: 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.

2.2. Storage

Store at room temperature (20–25°C).

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack Size Cat. No.	10 purifications Sample Pack	100 purifications 28-9034-70	250 purifications 28-9034-71
	Capture * buffer type 3	10 ml	55 ml	140 ml
	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)
	Elution ** buffer type 4	12 ml	12 ml	28 ml

* 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.

** 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.

Identification	Pack Size Cat. No.	10 purifications Sample Pack	100 purifications 28-9034-70	250 purifications 28-9034-71
	Elution * 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

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

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

GE 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.

3.2. 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

3.3. 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

4. Description

4.1. Introduction

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 µl 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).

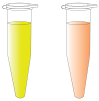
The Capture buffer type 3 contains a pH indicator that changes color at various pH levels to visually indicate whether the Capture buffer plus sample mix is at the optimal pH for DNA to bind to the silica membrane.

For efficient binding of DNA to the silica membrane the Capture buffer-sample mix requires a pH ≤ 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:

Optimal pH



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:

pH too high

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 µl elution volume. Table 1 opposite shows example percentage yields obtained when purifying a 910 bp fragment.

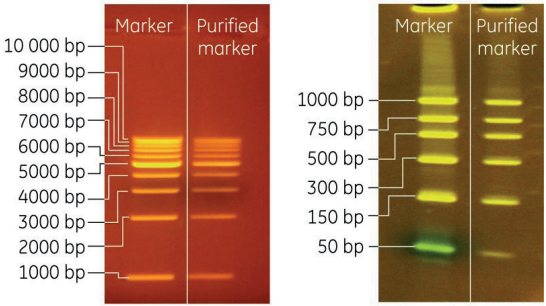
Table 1. Percentage yield obtained with illustra GFX PCR DNA and Gel Band Purification Kit

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

*910 bp PCR fragment at 8.4 ng/µl 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. Figure 1 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.

Figure 1: Agarose gel analysis of markers purified using the illustra GFX PCR DNA and Gel Band Purification Kit



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

Table 2 shows typical yield for fragments of differing sizes.

Table 2: Percentage yield obtained for fragments of differing sizes

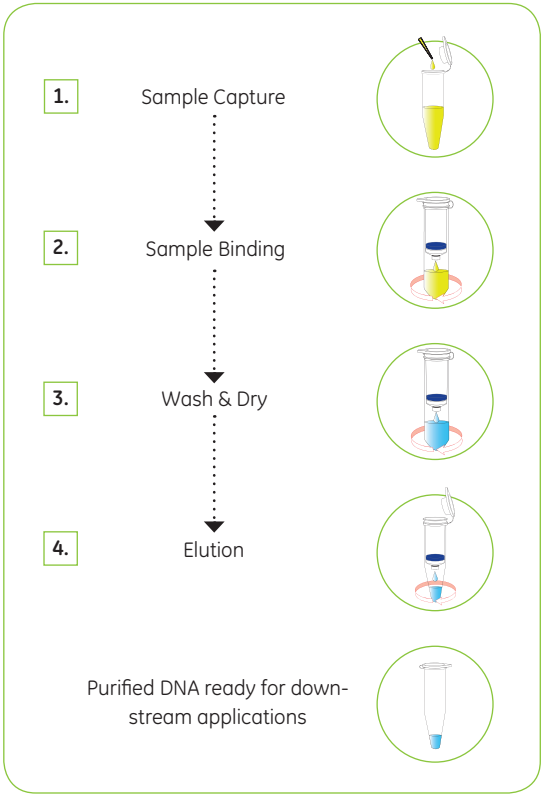
Fragment size	Percent recovery
250	83.7
500	84.7
1 000	94.7
1 500	91.8
2 000	91.1
3 000	95.4
4 000	86.4
6 000	68.1
10 000	42.6

10 µl of KiloBase DNA Marker was purified using the illustra GFX PCR DNA and Gel Band Purification Kit, and eluted with 50 µl of sterile DNase-free water. 10 µl of purified sample was loaded onto a 0.8% agarose gel. For comparison, unpurified marker was diluted 1:5 and 10 µl 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.

4.2. 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**
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**
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** ***OR*** **Elution buffer type 6**

4.3. 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 nglµl	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%

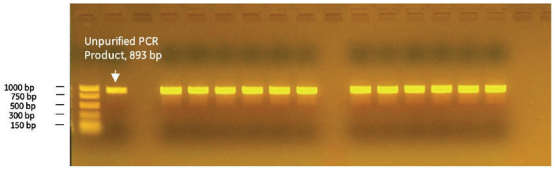
When purifying a PCR fragment less than 50 bp (but greater than 10 bases) in length, use an illustra MicroSpin G-25 Column.

This kit cannot be used for the purification of RNA.

If handling large numbers of samples in solution, 100 bp–10 kbp in length, use an illustra GFX 96 PCR Purification Kit.

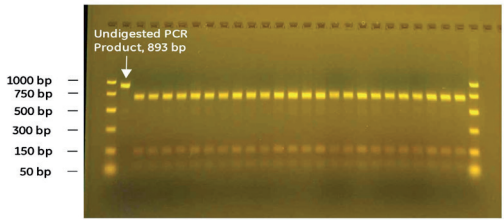
4.4. Typical output

Figure 2. Purified PCR product



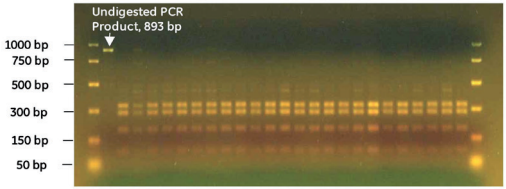
Purified p53 PCR fragments (893 bp), ~ 100 ng samples run on a 1% (w/v) agarose gel.

Figure 3. PCR product digested using MscI restriction enzyme



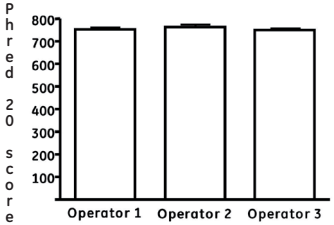
Purified p53 PCR fragments (893 bp) digested with the salt sensitive restriction enzyme MscI (200 ng plasmid DNA, 1 unit MscI, 37°C for 1 hour). Expected fragments from MscI digestion of p53 PCR fragment: 685 bp, 139 bp & 69 bp.

Figure 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.

Figure 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).

5. Protocol

Note: Solutions are NOT transferable between GE 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

The Key below describes the purpose of the icons used throughout the protocol booklet.

This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.

This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

5.1. Preparation of working solutions

See section 3.2 and 3.3 for Materials & Equipment to be supplied by user.

Capture buffer type 3

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 section 4.1 for more information.

Wash buffer type 1

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 28-9034-70 or add 250 ml ethanol to the Wash buffer type 1 in kit 28-9034-71. 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 (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 (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.

5.2. 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 5.4 Protocol for purification of DNA from TAE and TBE agarose gels.

5.3. Protocol for purification of DNA from solution or an enzymatic reaction

1. Sample Capture

a. Add 500 µl **Capture buffer type 3** to up to 100 µl sample.

500 µl Capture buffer type 3 to ≤ 100 µl sample

Note: If sample volume is greater than 100 µl, divide the sample and purify using more than one GFX MicroSpin column.

b. Mix thoroughly.

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

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

Note: If the color of the binding mixture is dark pink or red, add a small volume (~ 10 µl) 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 section 4.1 for more information.

d. For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.

2. Sample Binding

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

Pulse

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

Load Capture buffer type 3-sample mix

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

30 seconds 16 000 × g

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

d. Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube.

3. Wash & Dry

a. Add 500 µl **Wash buffer type 1** to the GFX MicroSpin column.

500 µl Wash buffer type 1

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

30 seconds 16 000 × g

Note: 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%.

c. Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 ml microcentrifuge tube (supplied by user).

4. Elution

a. Add 10–50 µl **Elution buffer type 4** *OR* **type 6** to the center of the membrane in the assembled GFX MicroSpin column and sample Collection tube.

10–50 µl Elution buffer type 4 ***OR*** type 6

b. Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute.

c. Spin the assembled column and sample Collection tube at 16 000 × g for 1 minute to recover the purified DNA.

1 minute RT

1 minute 16 000 × g

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

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

1. Sample Capture

a. Weigh a DNase-free 1.5 ml microcentrifuge tube and record the weight.

b. 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).

c. Weigh the microcentrifuge tube plus agarose band and calculate the weight of the agarose slice.

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

d. Add 10 µl **Capture buffer type 3** for each 10 mg of gel slice, for example, add 300 µl Capture buffer type 3 to each 300 mg gel slice.

10 µl Capture buffer type 3 per 10 mg gel slice

Note: If the gel slice weighs less that 300 mg, add 300 µl Capture buffer type 3. DO NOT add less than 300 µl Capture buffer type 3 per sample.

Note: To save time when purifying mulitple samples of gel bands (each weighing less than 500 mg), add 500 µl 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.

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

60°C until agarose dissolves

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

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

Note: If the color of the binding mixture is dark pink or red, add a small volume (~ 10 µl) 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 section 4.1 for more information.

g. For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.

2. Sample Binding

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

Pulse

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

Apply up to 800 µl Capture buffer type 3-sample mix

Note: the cap of the Collection tube can be used to cap the GFX Microspin column. If the cap is not required cut if off.

c. Incubate at room temperature for 1 minute.

1 minute RT

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

30 seconds 16 000 × g

e. Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube.

f. Repeat Sample Binding steps b. to e. as necessary until all sample is loaded.

3. Wash & Dry

a. Add 500 µl **Wash buffer type 1** to the GFX MicroSpin column.

500 µl Wash buffer type 1

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

30 seconds
16 000 × g

Note: 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%.

c. Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 ml microcentrifuge tube (supplied by user).

10–50 µl Elution buffer type 4 OR type 6

4. Elution

a. Add 10–50 µl **Elution buffer type 4** *OR* **type 6** to the center of the membrane in the assembled GFX MicroSpin column and sample Collection tube.

1 minute
RT

b. Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute.

1 minute
16 000 × g

c. Spin the assembled column and sample Collection tube at 16 000 × g for 1 minute to recover the purified DNA.

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

6. Appendix

6.1. RPM calculation from RCF.

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

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

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 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

6.2. Troubleshooting guide

This guide may be helpful in the first instance. However, if problems persist, or for further information, please contact GE technical services. Visit http://www.gelifesciences.com for contact information. Alternatively, log onto http://www.gehealthcare.com/illustra

Problem: DNA yield is low

Possible cause	Suggestions
Incorrect volume of Capture buffer type 3 used	• 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. • 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.

Problem: DNA yield is low *(Continued)*

Possible Cause	Suggestions
Wash buffer type 1 was not completely removed before Elution step	• The 30 second centrifugation at 16 000 × 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 × g.
Agarose gel band was not fully dissolved in the Capture buffer type 3	• 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.
Capture buffer type 3-sample mix was at pH > 7.5	• 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.

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

Possible Cause	Suggestions
Wash buffer type 1 was not completely removed before Elution step	• The 30 second centrifugation at 16 000 × 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 × g.

Problem: DNA sample floats out of well when loading a gel *(Cont)*

Possible Cause	Suggestions
Collection tube was not emptied after the centrifugation for the Sample Binding step. This can cause the Collection tube to overfill and the Wash & Dry step to be affected.	• Always empty the Collection tube after the centrifugation within the Sample Binding step.

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

Possible cause	Suggestions
Incorrect Elution buffer used	• 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.
Sample was not present as a single band prior to purification	• 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 5.4 Protocol for purification of DNA from TAE and TBE agarose gels.

Problem: DNA fails to ligate

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

6.3. Related products

A full range of Molecular Biology reagents can be found on the GE web site and in the catalog.
http://www.gehealthcare.com/illustra

If you need further information, GE technical services are happy to assist. Visit http://www.gelifesciences.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	28-9034-45	10 × 96 well plates
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)	27-5325-01	50 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50 Columns	27-5340-01	50 purifications

Application	Product	Product code	Pack size
Preparation of circular DNA templates	illustra TempliPhi™ 100 Amplification Kit	25-6400-10	100 reactions
Genomic DNA amplification	illustra GenomiPhi™ V2 DNA Amplification Kit	25-6600-30	25 reactions
	illustra GenomiPhi HY DNA Amplification Kit	25-6600-22	25 reactions
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25-1500-01	100 reactions
	illustra PuReTaq Ready-To-Go™ PCR Beads	27-9557-01	96 reactions in 0.2 ml tubes/ plate
	illustra PuReTaq Ready-To-Go PCR Beads	27-9557-02	5 × 96 reactions in 0.2 ml tubes/ plate
	FideliTaq PCR Master Mix Plus (2 ×)	E71182	100 reactions
	FideliTaq Master Mix Plus	E71183	100 reactions

Application	Product	Product code	Pack size
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix dNTP Set (A,C,G,T) 20 mM each	28-4065-57	10 µmol
	illustra DNA Polymerization Mix dNTP Set (A,C,G,T) 20 mM each	28-4065-58	40 µmol (4 × 10 µmol)
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 25 mM each	28-4065-60	500 µl
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 2 mM each	28-4065-62	1 ml

7.0. References

1. Vogelstein, B. & Gillespie, D., *Proc. Natl. Acad. Sci. USA* 76, 615 (1979).

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

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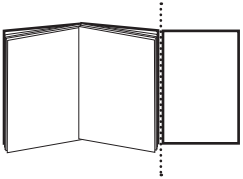
The next four pages are protocol cards.
Please add to the back page as a tear off addition.



Quick Reference Protocol Card

illustra™ GFX™ PCR DNA and Gel Band Purification Kit

28-9034-70 (100 purifications)
28-9034-71 (250 purifications)

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

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

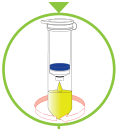
:Add :Spin :Incubate

1. Sample capture

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

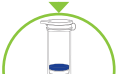
2. Sample binding

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



3. Wash & dry

- 500 µl Wash buffer type 1
- 30 s 16 000 × g



- Discard Collection tube. Transfer GFX MicroSpin column to a clean 1.5ml DNase-free microcentrifuge tube.

4. Elution

OR 10–50 µl Elution buffer type 4 OR type 6

60 s room temperature

60 s 16 000 × g

- Retain flow through
- Store purified sample DNA at -20°C

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Buckinghamshire, HP7 9NA UK

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Quick Reference Protocol Card

illustra™ GFX™ PCR DNA and Gel Band Purification Kit

28-9034-70 (100 purifications)
28-9034-71 (250 purifications)

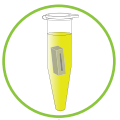
B. Protocol for purification of DNA from TAE and TBE agarose gels

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

:Add :Spin :Incubate

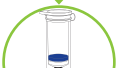
1. Sample capture

- 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 µl 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 µl Capture buffer type 3-sample mix to assembled GFX MicroSpin™ column and Collection tube
- 60 s room temperature



30 s 16 000 × 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 µl Wash buffer type 1

30 s 16 000 × g

- Discard Collection tube. Transfer GFX MicroSpin column to a clean 1.5ml DNase-free microcentrifuge tube.

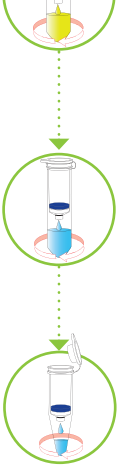
4. Elution

OR 10–50 µl Elution buffer type 4 OR type 6

60 s room temperature

60 s 16 000 × g

- Retain flow through
- Store purified sample DNA at -20°C



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