GE Healthcare

illustra GFX 96 PCR Purification Kit

For the parallel purification of multiple PCR products

Product booklet

Code: 28-9034-45 (10 × 96 well plates)

Page finder

1. Legal

Product use restriction

The components of the **illustra™ GFX™ 96 PCR 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 96 PCR Purification Kit** for a specific application as the performance characteristics of this kit have not been verified for any specific organism.

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http://www.gelifesciences.com

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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 the 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

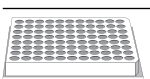
Store at room temperature (20–25°C).

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack Size Cat. No.	2 × 96 well plates Sample Pack	10 × 96 well plates 28-9034-45
	Capture buffer type 2 (Blue colored cap)	60 ml	288 ml
	Wash buffer type 1 (Yellow colored cap)	62.5 ml (Add 250 ml absolute ethanol before use)	80 ml (Add 350 ml absolute ethanol before use)
	Elution buffer type 4* (Silver colored cap)	12 ml	48 ml
	Elution buffer type 6* (Pink colored cap)	12 ml	48 ml
	illustra™ GFX™Binding Plate	2	10
	illustra GFX Wash Plate	2	10
	illustra GFX Collection Plate	2	10

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

Elution buffer type 4 can be used to elute samples for multiple downstream applications and should be used for samples requiring long term storage. **Elution buffer type 6** should be used for samples to be sequenced only, especially if using a capillary loading analyzer.

GE Healthcare 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 96 PCR 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 capture, Wash and Elution buffer for your purification.

3.2. Materials to be supplied by user

Disposables:
Reagent reservoir

Chemicals:
Absolute ethanol

3.3. Equipment needed

Bench top microcentrifuge suitable for centrifugation of 96 well plates
OR
NucleoVac™ 96 Vacuum Manifold, available from Macherey-Nagel (product code 740681).

4. Description

4.1. Introduction

The **illustra GFX 96 PCR Purification Kit** is designed for the purification and concentration of DNA from PCR mixtures, restriction enzyme digestions and solutions. DNA ranging in size from 100 bp up to 10 kbp can be purified from solution volumes of up to 300 µl.

The illustra GFX 96 PCR Purification Kit utilizes glass fiber matrix technology in a 96 well format. DNA fragments are captured by the matrix in the presence of a chaotropic salt, and contaminants are removed by washing the matrix with a buffered ethanol solution. Purified DNA is recovered by elution in a small volume of water or a low ionic strength buffer. The purified DNA is ready for use in a variety of applications, including fluorescent sequencing, labeling, hybridization, ligation and transformation.

The illustra GFX 96 PCR Purification Kit provides Binding, Wash and Collection plates and all required buffers in one box. The standard configuration of the 96 well plates is compatible with most 96 well plate adapted benchtop centrifuges and the NucleoVac 96 Vacuum Manifold from Macherey-Nagel. There are no hazardous organic extractions, and ethanol precipitation is not required to isolate purified DNA. The kit allows 96 PCR products to be purified in as little as 15 minutes.

Typical reactions from which DNA can be isolated include:

- PCR mixtures.
- Sequential restriction enzyme digests, where the enzymes involved have differing buffer requirements; perform the first digest, purify the sample using the **illustra GFX 96 PCR 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.

Table 1 below shows typical percentage yields obtained when purifying a 910 bp fragment.

Table 1: Percentage yield obtained with illustra GFX 96 PCR Purification Kit

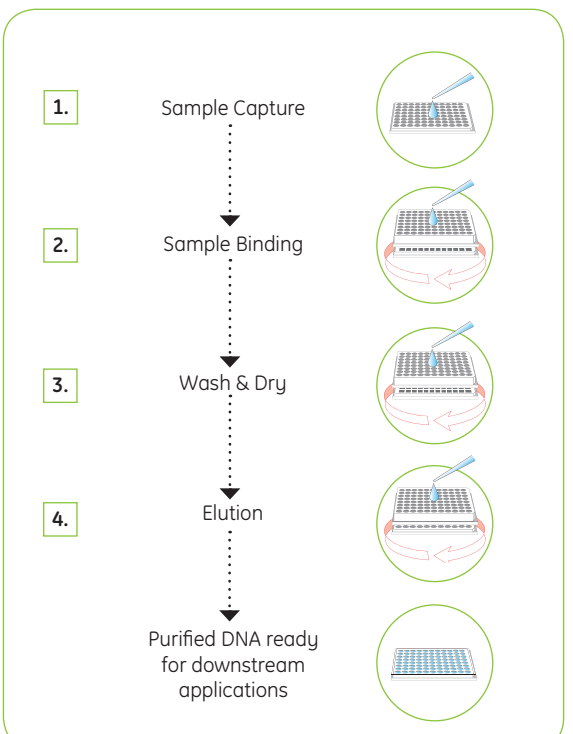
Protocol	Elution volume (µl)	Yield (%)
Centrifugation*	10	55
	50	95
	100	90
Vacuum†	50	59
	100	78
Vacuum plus optional additional Wash & Dry step	50	51

* For centriguation protocol, see section 5.3.

† For vacuum protocol, see section 5.4.

4.2. The basic principle

Use of the **illustra GFX 96 PCR Purification Kit** involves use of a benchtop centrifuge or vacuum for the following steps (images have been shown for the centrifugation protocol only and are intended to indicate the addition of sample or buffer to all 96 wells using a robot or multichannel pipette):



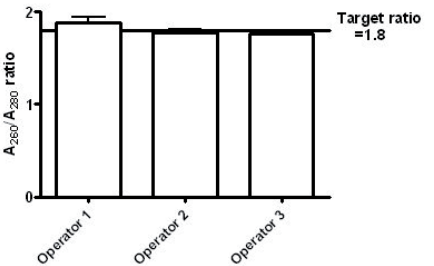
Step	Comments	Component
1. Sample Capture	Capture buffer type 2 is added to the samples. Proteins are denatured.	**Capture buffer type 2** 
2. Sample Binding	The Capture buffer type 2 - sample mix is applied to the illustra GFX Binding Plate assembled on the illustra GFX Wash Plate DNA binds to the membrane.	**illustra GFX Binding Plate & illustra GFX Wash Plate** 
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. Elute sample with **Elution buffer type 4** for multiple downstream applications and long term storage of samples. Use **Elution buffer type 6** for samples to be sequenced only.	**illustra GFX Binding Plate & illustra GFX Collection Plate**  **Elution buffer type 4**  **OR** **Elution buffer type 6** 

4.3. Product specifications

Sample type	**PCR mixtures, enzyme reactions & DNA solutions**
Sample size range	10 kbp–100 bp
Input volume	Up to 100 µl solution
Elution volume	50–100 µl (into one of two elution buffers provided)
Major subsequent applications	Further PCR amplification, sequencing, labeling, restriction enzyme digestion, ligation, cloning.
Yield	Up to 95% when purifying a 910 bp fragment at a starting concentration of 8.4 ng/µl.
Protocol length	15 minutes for multiple samples
Protocol type	Vacuum or centrifuge

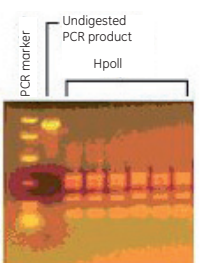
4.4. Typical output

Figure 1: A_{260}/A_{280} as an indicator of sample purity



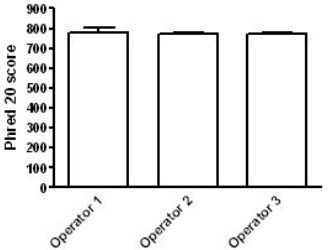
Three separate operators purified 6 PCR mixtures. Purity was measured by absorbance at 260 and 280 nm.

Figure 2: Restriction enzyme digestion as an indication of sample purity



Typical gel image obtained when using purified P53 Tumor Protein PCR product for restriction enzyme digestion with the salt sensitive enzyme HpaII. (Marker sizes 1 000, 750, 500, 300, 150 & 50 bp. Expected digested fragment sizes, 321, 283, 187 & 102 bp).

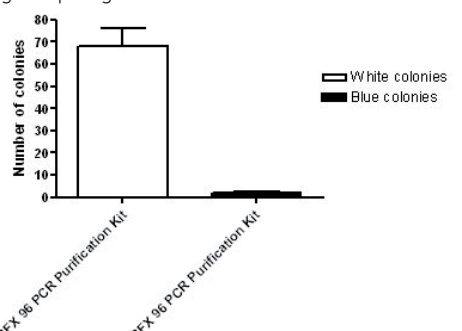
Figure 3: Phred 20 scores as an indicator of sample purity



DNA sequencing	Mean Phred 20 read length
Operator	illustra
1	780
2	774
3	770

Three separate operators sent 12 purified PCR samples to a sequencing service provider, who performed all sequencing reactions and analysis.

Figure 4: Ligation and cloning results as an indication of sample quality and purity



18 purified PCR products were used in ligation and transformation reactions with a pUC18-based vector and a TA cloning kit. Blue/white selection was used to indicate recombinant clones. The PCR product was amplified from an ampicillin resistant plasmid, but kanamycin was used as the selectable marker during the cloning step to eliminate the risk of plasmid carry over.

5. Protocols

Note: Solutions are NOT transferable between GE Healthcare illustra kits e.g. the composition of the Capture buffer in the GFX 96 PCR 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.

Wash buffer type 1

Prior to first use, add absolute ethanol to the bottle containing Wash buffer type 1. Add 350 ml of absolute ethanol to the Wash buffer type 1 in kit 28-9034-45. Mix by inversion. Indicate on the label that this step has been completed. Store upright and airtight.

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  -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  -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 Preparation of vacuum source

If required, the GFX 96 PCR Purification Kit can be used with the Macherey-Nagel NucleoVac 96 vacuum manifold. To process less than 96 samples use a rubber pad or self-adhering PE foil to cover up any unused wells of the GFX 96 Binding Plate and ensure a vacuum is maintained.

Establish a reliable vacuum source for the manifold. A vacuum pump, house vacuum or water aspirator may be used. We recommend a vacuum of 380–630 mbar or 15–25 mm Hg. The use of a vacuum regulator is recommended. Alternatively, adjust the vacuum so that during purification the sample flows through the column at a rate of 1–2 drops per second. Vacuum times suggested in protocol 5.4 might have to be increased for complete filtration if large sample volumes are used.

5.3 Centrifugation protocol for purification of DNA from solution or an enzymatic reaction

Images are intended to indicate the addition of sample or buffer to all 96 wells using a robot or multichannel pipette.

1. Sample Capture

a. Add approximately 30 ml **Capture buffer type 2** to a reagent reservoir.

b. Use a multichannel pipette to add 300 µl **Capture buffer type 2** to each sample to be purified.

Note: Sample volume may be 20–300 µl.

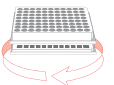
c. Mix well.

d. Assemble the GFX Binding Plate on top of the GFX Wash Plate.

2. Sample Binding

a. Add the **Capture buffer type 2**-sample mix to the GFX Binding Plate.

b. Centrifuge the assembled plates for 2 minutes at 1 800 × g.

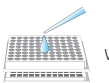


c. Discard the flowthrough by emptying the GFX Wash Plate. Re-assemble the GFX Binding Plate onto the same GFX Wash Plate.

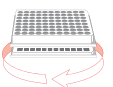
3. Wash & Dry

a. Add approximately 40 ml **Wash buffer type 1** to a fresh reagent reservoir.

b. Use a multichannel pipette to add 400 µl **Wash buffer type 1** to each well of the GFX Binding Plate.



c. Spin the assembled plates for 5 minutes at 1 800 × g.



Note: Wash & Dry steps a.-c. may be repeated when purity is paramount, for example if the sample is to be used in a blunt-ended ligation.

d. Discard the flowthrough by emptying the GFX Wash Plate. Re-assemble the GFX Binding Plate onto the same GFX Wash Plate.

e. Centrifuge the assembled plates for an additional 30 seconds at 1 800 × g.

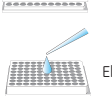
Note: Steps d. and e. are vital to remove residual ethanol and ensure good performance in downstream applications.

4. Elution

a. Discard the GFX Wash Plate.

b. Assemble the GFX Binding Plate onto the GFX Collection Plate.

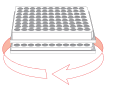
c. Add 50–100 µl **Elution buffer type 4** OR **type 6** to the center of the membrane in each well of the GFX Binding Plate.



d. Incubate for 1 minute at room temperature (RT).

1 minute
RT

e. Spin assembled plates for 2 minutes at 1 800 × g to recover the purified DNA in the GFX Collection Plate.



2 minutes
1 800 × g

Note: When 50 µl volume is used for elution, an average volume of 45–48 µl will be recovered.

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

5.4 Vacuum protocol for purification of DNA from solution or an enzymatic reaction

Images are intended to indicate the addition of sample or buffer to all 96 wells using a robot or multichannel pipette.

1. Sample Capture

a. Add approximately 30 ml **Capture buffer type 2** to a reagent reservoir.

b. Use a multichannel pipette to add 300 µl **Capture buffer type 2** to each sample to be purified.

300 µl Capture buffer type 2 to 20–300 µl sample

Note: Sample volume may be 20–300 µl.

c. Mix well.

d. Place the GFX Wash Plate in the bottom of the manifold. Close the manifold and place the GFX Binding Plate on top.

2. Sample Binding

a. Add the **Capture buffer type 2**-sample mix to the GFX Binding Plate.

b. Apply vacuum (380–630 mbar or 15–25 mm Hg) for 3 minutes.

3 minutes
380–630 mbar
or
15–25 mm Hg

c. Turn off the vacuum. Discard the flowthrough by emptying the GFX Wash Plate. Re-assemble the GFX Binding Plate onto the same GFX Wash Plate within the manifold.

3. Wash & Dry

a. Add approximately 40 ml **Wash buffer type 1** to a fresh reagent reservoir.

b. Use a multichannel pipette to add 800 µl **Wash buffer type 1** to each well of the GFX Binding Plate.

800 µl Wash
buffer type 1

c. Apply vacuum (380–630 mbar or 15–25 mm Hg) for 10 minutes.

10 minutes
380–630 mbar
or
15–25 mm Hg

Note: Wash & Dry steps a.-c. can be repeated when purity is paramount, for example if the sample is to be used in a blunt-ended ligation.

d. Turn off the vacuum. Withdraw the GFX Binding Plate from the manifold. Gently tap the bottom of the plate 3 times onto a stack of lint-free tissues to remove excess **Wash buffer type 1** from the plate's nozzles.

4. Elution

a. Discard the GFX Wash Plate.

b. Place the GFX Collection Plate in the bottom of the manifold. Close the manifold and place the GFX Binding Plate on top.

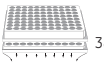
c. Add 50–100 µl **Elution buffer type 4** **OR type 6** to the center of the membrane in each well of the GFX Binding Plate.

50–100 µl Elution buffer type 4 OR type 6

d. Incubate for 1 minute at room temperature (RT).

1 minute RT

e. Apply vacuum (380–630 mbar or 15–25 mm Hg) for 5 minutes.



5 minutes 380–630 mbar or 15–25 mm Hg

Note: When 50 µl volume is used for elution, an average volume of 45–48 µl will be recovered.

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

6. Appendices

6.1. RPM calculation from RCF

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

RPM=1 000 × √(RCF/1.12r)

Where RCF = relative centrifugal force; r = radius in mm measured from the center 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 Healthcare technical services. Telephone numbers are on the back page. Alternatively log onto http://www.gelifesciences.com/illustra.

Problem: plasmid DNA yield is low

Possible cause	Suggestions
Incorrect volume of Capture buffer type 2 used	• Check that the volumes of sample and Capture buffer type 2 are correct (300 µl Capture buffer type 2 and 20–300 µl sample)
Wash buffer type 1 was not completely removed before Elution step	• Centrifugation protocol - the 30 second centrifugation at 1 800 × g to dry the GFX Binding Plate during the Wash & Dry step is vital. • Vacuum protocol - Drying the GFX Binding Plate on a stack of lint-free tissues during the Wash & Dry step is vital.

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	• Centrifugation protocol- the 30 second centrifugation at 1 800 × g to dry the GFX Binding Plate during the Wash & Dry step is vital. • Vacuum protocol-drying the GFX Binding Plate on a stack of lint-free tissues during the Wash & Dry step is vital.

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 use an illustra GFX PCR DNA and Gel Band Purification Kit to isolate your DNA.

Problem: DNA fails to ligate

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

6.3. Related products

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

If you need further information, GE technical services are happy to assist (world-wide phone numbers can be found on the back cover).

Application	Product	Product code	Pack size
Purification of DNA from PCR, agarose gel bands and enzymes	illustra GFX PCR DNA & Gel Band Purification Kit	28-9034-70	100 purifications
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	25-6600-25	100 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
DNA ligation	DNA ligation System	RPN1507	50 reactions
	Ligate-IT Rapid Ligation Kit	US78400	25 reactions
	Ready-To-Go T4 DNA ligase	27-0361-01	50 reactions
Blunt-ended PCR cloning	Blunt-ended PCR Cloning Kit	RPN5110	40 reactions

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imagination at work

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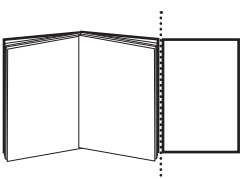
The next four pages are a protocol card.
Please add to the back page as a tear off addition.



Quick Reference Protocol Card

28-9034-45 (10 × 96 well plates)

illustra™ GFX™ 96 PCR Purification Kit

A.Centrifugation protocol for purification of DNA from solution or an enzymatic reaction

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

:Add :Spin :Incubate

1. Sample Capture

300 µl Capture buffer type 2 to each 20–600 µl sample; mix well

- **Assemble GFX Binding Plate and GFX Wash Plate**

2. Sample Binding

- **Load Capture buffer type 2-sample mix onto the GFX Binding Plate**

2 minutes 1 800 × g

- **Discard flowthrough**

3. Wash & Dry

400 µl Wash buffer type 1

5 minutes 1 800 × g

- Discard flowthrough

30 seconds 1 800 × g

4. Elution

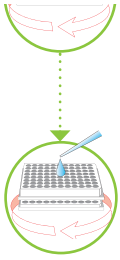
- Assemble GFX Binding Plate onto GFX Collection Plate

50–100 µl Elution buffer type 4 OR type 6.

1 minute at room temperature

2 minutes 1 800 × g; retain eluate

- Store purified DNA at -20°C



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

Quick Reference Protocol Card

28-9034-45 (10 × 96 well plates)

illustra™ GFX™ 96 PCR Purification Kit

B. Vacuum protocol for purification of DNA from solution or an enzymatic reaction

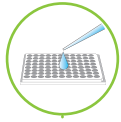
- Check appropriate volume of ethanol added to **Wash buffer type 1**

:Add :Spin :Incubate

1. Sample Capture

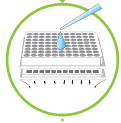
300 µl Capture buffer type 2 to each 20–600 µl sample; mix well

- **Assemble GFX Binding Plate and GFX Wash Plate within manifold**



2. Sample Binding

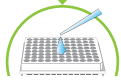
- **Load Capture buffer type 2-sample mix onto the GFX Binding Plate**
- **Apply vacuum 3 minutes 380–630 mbar or 15–25 mm Hg**
- **Discard flowthrough**



3. Wash & Dry

400 µl Wash buffer type 1

- **Apply vacuum 10 minutes 380–630 mbar or 15–25 mm Hg**



- Discard flowthrough
- Dry GFX Binding Plate on a stack of lint-free tissues

4. Elution

- Assemble GFX Binding Plate onto GFX Collection Plate in the manifold

50–100 µl Elution buffer type 4 OR type 6

1 minute at room temperature

- Apply vacuum 5 minutes 380–630 mbar or 15–25 mm Hg
- Store purified DNA at -20°C