GE Healthcare

illustra CyScribe GFX Purification kit

For the purification of CyDye labeled and unlabeled cDNA.

Product booklet

Codes: 27-9606-01 (25 purifications)
27-9606-02 (50 purifications)

Page finder

1. Legal

Product use restriction

The **illustra™ CyScribe™ GFX™ Purification Kit** and components 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 their 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 CyScribe GFX Purification Kit** for a specific application, as the performance characteristics of this product have not been verified for any specific organism.

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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 the Capture buffer type 1 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

All kit components should be stored 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.	25 purifications 27-9606-01	50 purifications 27-9606-02
	Capture buffer type 1 (Blue colored cap)	12.5 ml	25 ml
	Wash buffer type 3 (Yellow colored cap)	15 ml (Add 50 ml Absolute Ethanol before use)	30 ml (Add 100 ml Absolute Ethanol before use)
	Elution buffer type 7 (Silver colored cap)	8 ml	16 ml
	illustra CyScribe GFX MicroSpin™ columns	25	50
	Collection tubes	50	50

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

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 CyScribe GFX Purification Kit is not the same as the Capture buffer supplied in the illustra GFX PCR DNA and Gel Band 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.

Note that the Cyscribe GFX MicroSpin columns 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 Cyscribe GFX Microspin column when it is inserted into the Collection tube.

3.2. Materials to be supplied by user

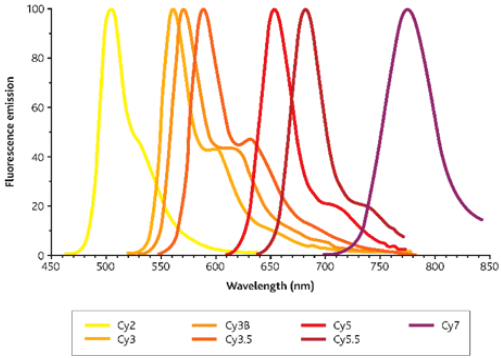
Disposables:
DNase-free 1.5 ml microcentrifuge tubes (snap cap). One microcentrifuge tube is required per sample if purifying labeled cDNA synthesized using the Amersham™ CyScribe First-Strand cDNA Labeling Kit. Two microcentrifuge tubes are required per sample if purifying and coupling CyDye to cDNA synthesized using the Amersham CyScribe Post-Labeling Kit. Amber tubes are recommended, as CyDye™ labeled cDNA should be stored in the dark.

Chemicals:
(see section 5.1 for preparation details)
To purify CyDye first-stand labeled probes:
Absolute Ethanol
2.5 M NaOH
2 M HEPES free acid
To purify CyDye post-labeled probes:

Absolute Ethanol
2.5 M NaOH
2 M HEPES free acid
80% Ethanol (v/v in water)
0.1 M Sodium Bicarbonate pH 9.0
4 M Hydroxylamine
For preparation details of all buffers please see section 5.1 Preparation of working solutions.

CyDye Post-Labeling Reactive Dye Pack (as appropriate for your downstream application) (These are supplied ready to use, individually dispensed in foil packs for protective storage; each vial is sufficient for one labeling reaction of up to 1 µg cDNA)

Figure 1. Emission Spectra of CyDye Fluorescent Dyes



3.3. Equipment needed

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes and capable of generating a g force of 13 800.
Vortex mixer
Water bath or heat block set at 37°C

4. Description

4.1. Introduction

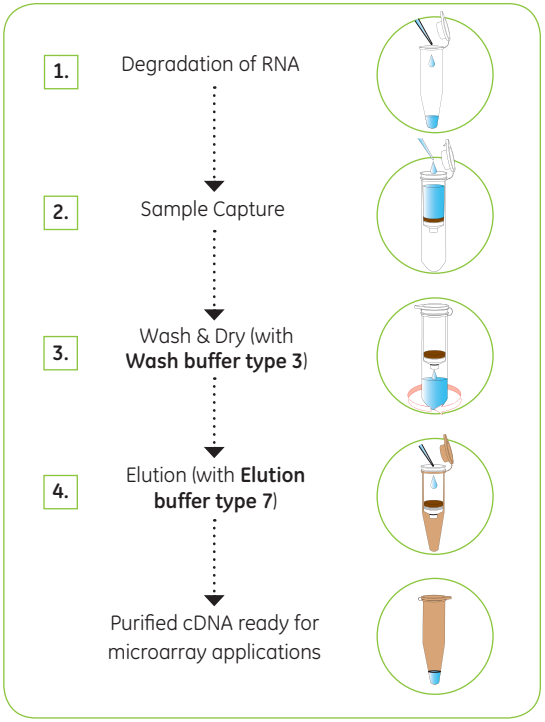
The **illustra CyScribe GFX Purification Kit** is designed for the rapid purification of cDNA prepared by the use of **Amersham CyScribe First-Strand cDNA Labeling Kit** and the **Amersham CyScribe Post-Labeling Kit**.

The **Amersham CyScribe First-Strand cDNA Labeling Kit**, containing CyScribe reverse transcriptase, can be used to prepare cDNA. Cy™3 or Cy5 dCTP or dUTP is used to directly label the synthesized cDNA. Protocol 5.2 should be followed to purify this cDNA in readiness for microarray.

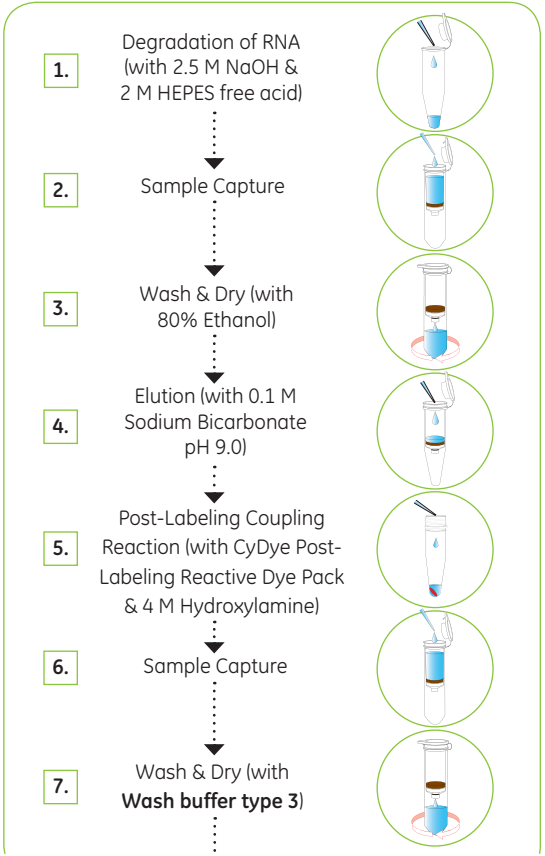
Use of the **Amersham CyScribe Post-Labeling Kit** features a longer protocol to prepare cDNA labeled with CyDye fluors. First, the Amersham CyScribe Post-Labeling Kit is used to synthesize first-stand cDNA incorporating a chemically reactive nucleotide analogue (aminoallyl-dUTP). The mRNA template is degraded and free nucleotides and oligomers removed by the CyScribe GFX Purification Kit (this kit). The purified cDNA is then "post-labeled" with the reactive forms of Cy3 or Cy5 - NHS esters, which bind to the reactive nucleotide analogues incorporated during cDNA synthesis. This is called the "post labeling coupling reaction". A second purification is performed with the CyScribe GFX Purification Kit (this kit). A full protocol detailing all these steps can be found in Section 5.3. Please make sure that you prepare the additional buffers required for purification of probes prepared with the Amersham CyScribe Post-Labeling Kit, as detailed in section 3.2.

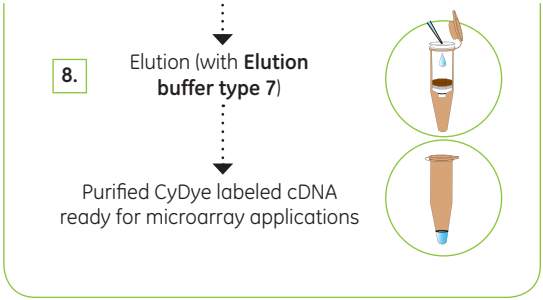
4.2. The basic principle

Use of the **illustra CyScribe GFX Purification Kit** involves the following steps when purifying cDNA from the **Amersham CyScribe First-Stand cDNA Labeling Kit**:



Use of the **illustra CyScribe GFX Purification Kit** involves the following steps when purifying cDNA from the **Amersham CyScribe Post-Labeling Kit:**





Steps 6–8 when working with post-labeled probes are identical to steps 2–4 when working with first-strand labeled probes.

Use of the **illustra CyScribe GFX Purification Kit** involves the following steps when purifying CyDye labeled cDNA synthesized with the **Amersham CyScribe First-Stand cDNA Labeling Kit**:

Step	Comments	Component
1. Degradation of RNA	RNA is degraded, leaving cDNA ready for purification	2.5 M NaOH & 2 M HEPES free acid
2. Sample Capture	Capture buffer type 1 is added to the sample. The Capture buffer type 1-sample mix is applied to the illustra CyScribe GFX MicroSpin column; DNA binds to the membrane.	**Capture buffer type 1**  **illustra CyScribe GFX MicroSpin column** 

Step	Comments	Component
3. Wash & Dry (with **Wash buffer type 3**)	A combined washing/ drying step removes salts and other contaminants from the membrane bound cDNA.	**Wash buffer type 3** 
4. Elution (with **Elution buffer type 7**)	Purified sample is eluted from the column with a low ionic strength buffer.	**Elution buffer type 7** 

Use of the **illustra CyScribe GFX Purification Kit** involves the following steps when purifying cDNA synthesized with the **Amersham CyScribe Post-Labeling Kit:**

Step	Comments	Component
1. Degradation of RNA (with 2.5 M NaOH & 2 M HEPES free acid)	RNA is degraded, leaving cDNA ready for purification.	2.5 M NaOH & 2 M HEPES free acid
2. Sample Capture	Capture buffer type 1 is added to the sample. The Capture buffer type 1 -sample mix is applied to the illustra CyScribe GFX MicroSpin column; DNA binds to the membrane.	**Capture buffer type 1**  **illustra CyScribe GFX MicroSpin column** 
3. Wash & Dry (with 80% Ethanol)	A combined washing/ drying step removes salts and other contaminants from the membrane bound cDNA. A wash step is performed with 80% (v/v) Ethanol.	80% Ethanol (v/v)

Step	Comments	Component
4. Elution (with 0.1 M Sodium Bicarbonate pH 9.0)	Purified sample is eluted from the column with 0.1 M Sodium Bicarbonate pH 9.0.	0.1 M Sodium Bicarbonate pH 9.0
5. Post-Labeling Coupling Reaction (with CyDye Post-Labeling Reactive Dye Pack & 4 M Hydroxylamine)	Cy3 or Cy5 NHS esters bind to the modified nucleotides in the cDNA	4 M Hydroxylamine
6. Sample Capture	Capture buffer type 1 is added to the sample. The Capture buffer type 1 -sample mix is applied to the illustra CyScribe GFX column; CyDye labeled cDNA binds to the membrane.	**Capture buffer type 1**  **illustra CyScribe GFX MicroSpin column** 
7. Wash & Dry (with **Wash buffer type 3**)	A combined washing/drying step removes salts and other contaminants from the membrane bound cDNA.	**Wash buffer type 3**
8. Elution (with **Elution buffer type 7**)	Purified CyDye labeled cDNA is eluted from the column with a low ionic strength buffer.	**Elution buffer type 7**

4.3. Product specifications

Sample type:	CyDye labeled cDNA synthesized with the Amersham CyScribe First-Strand cDNA Labeling Kit	cDNA and CyDye labeled cDNA synthesized with the Amersham CyScribe Post-Labeling Kit
Input sample volume	20–100 µl	20–100 µl
Size range of fragments purified	> 50 bp	> 50 bp
Major subsequent application	Microarray hybridization	Microarray hybridization
Number of steps	4	8
Total time for full protocol	< 30 minutes per 2 purifications	< 2.5 hours per 2 purifications

This kit is not suitable for the purification of RNA.

4.4. Typical output

The data below has been generated using the Amersham CyScribe Post-Labeling Kit in combination with the illustra CyScribe GFX Purification Kit. The cDNA was generated using the protocol provided with the Amersham CyScribe Post-Labeling Kit. For data in **Tables 1a, 1b, & 1c**. 1 µg of **mRNA** was used for cDNA synthesis using the conditions indicated. For data in **Table 2a, 2b, & 2c**, 5 µg of **total RNA** was used for cDNA synthesis.

Table 1a. Typical yield and purity when purifying cDNA generated from mRNA using the conditions below

Mean yield cDNA (ng/µl) after step 4 Elution (with 0.1 M Sodium Bicarbonate pH 9.0)	Mean A_{260}/A_{280}
6.2	1.9

n=3

cDNA generation conditions: 1 µg mRNA plus 1 µl random nonamer and 1 µl oligo d(T); 1.5 hours incubation.

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

Table 1b. Results obtained with 50 µl of sample from Table 1a. in a Cy3 Post-labeling Coupling Reaction and subsequent purification with CyScribe GFX Purification kit

Yield CyDye labeled cDNA (ng/µl) after step 8 Elution (with **Elution buffer type 7**)	Mean A_{260}/A_{280}	Cy3 pmol/µg cDNA	Cy3 FOI
3.73	2.63	129.3	42

n=3

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

Table 1c. Results obtained with 50 µl of sample from Table 1a. in a Cy5 Post-labeling Coupling Reaction and subsequent purification with CyScribe GFX Purification kit

Yield CyDye labeled cDNA (ng/µl) after step 8 Elution (with **Elution buffer type 7**)	Mean A_{260}/A_{280}	Cy5 pmol/µg cDNA	Cy5 FOI
2.58	2.96	86	28

n=3

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

Table 2a. Typical yield and purity when purifying cDNA generated from total RNA using the conditions below

Mean yield cDNA (ng/µl) after step 4 Elution (with 0.1 M Sodium Bicarbonate pH 9.0)	Mean A_{260}/A_{280}
14.3	1.76

n=3

cDNA generation conditions: 5 µg total RNA plus 1 µl random nonamer and 1 µl oligo d(T); 1.5 hours incubation.

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

Table 2b. Results obtained with 50 µl of sample from Table 1a. in a Cy3 Post-labeling Coupling Reaction and subsequent purification with CyScribe GFX Purification kit

Yield CyDye labeled cDNA (ng/µl) after step 8 Elution (with Elution buffer type 7)	Mean A_{260}/A_{280}	Cy3 pmol/µg cDNA	Cy3 FOI
6.59	1.73	139.3	45

n=3

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

Table 2c. Results obtained with 50 µl of sample from Table 2a. in a Cy5 Post-labeling Coupling Reaction and subsequent purification with CyScribe GFX Purification kit

Yield CyDye labeled cDNA (ng/µl) after step 8 Elution (with Elution buffer type 7)	Mean A_{260}/A_{280}	Cy5 pmol/µg cDNA	Cy5 FOI
6.28	2.11	118	38.3

Elution conditions: Two × 60 µl of Sodium Bicarbonate with 5 minutes incubation time each.

5. Protocol

Note: Solutions and MicroSpin columns are NOT transferable between GE Healthcare kits e.g., the composition of the Wash Buffer in the CyScribe GFX Purification Kit is not the same as the Wash Buffer in the GFX PCR DNA and Gel Band Purification Kit, and the illustra MicroSpin columns supplied in the CyScribe GFX Purification Kit are not the same as the columns provided in the GFX PCR DNA and Gel Band 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 3

Prior to first use, add Absolute Ethanol to the bottle containing Wash buffer type 3. Add 50 ml of Absolute Ethanol to the Wash buffer type 3 in kit 27-9606-01 or add 100 ml Absolute Ethanol to the Wash buffer type 3 in kit 27-9606-02. Mix by inversion. Indicate on the label that this step has been completed. Store upright with the cap tightly closed.

2.5 M NaOH

Consult MSDS supplied with sodium hydroxide pellets for safe handling of this compound.

Add 1 g NaOH to 8 ml sterile distilled DNase-free water. Bring to a final volume of 10 ml with sterile distilled DNase-free water. Filter sterilize the solution with a 0.45 µm filter. Store at ambient temperature. For best results use fresh 2.5 M NaOH; it is advisable not to store this solution beyond 3 months.

2 M HEPES free acid

It is recommended that a DNase and RNase free source of HEPES is used.

Dissolve 4.77 g HEPES free acid in 9 ml sterile distilled DNase-free water. Make volume up to 10 ml with sterile distilled DNase-free water. Filter sterilize the solution with a 0.45 µm filter. Store at ambient temperature. For best results use fresh 2 M HEPES; it is advisable not to store this solution beyond 3 months.

80% (v/v) Ethanol.

To 80 ml Ethanol add 20 ml sterile distilled DNase-free water. Store at room temperature. Once made up, would be stable past expiry date of kit, unless local conditions are particularly warm. Store upright in an airtight container.

0.1 M Sodium Bicarbonate pH 9.0

For 100 ml, dissolve 0.84 g Sodium Bicarbonate salt in 90 ml of sterile distilled DNase-free water. Adjust to pH 9.0 with 1 M NaOH. Bring to a final volume of 100 ml with sterile DNase-free water. Filter sterilize the solution with a 0.45 µm filter. Dispense into aliquots and store at -20°C for up to three months.

4 M Hydroxylamine

Consult MSDS supplied with Hydroxylamine for safe handling of this compound.

For example, dissolve 2.78 g Hydroxylamine Hydrochloride NH_2OH. HCl in 9 ml sterile distilled DNase-free water. Bring to a final volume of 10 ml with sterile distilled DNase-free water. Filter sterilize with a 0.45 µm filter.

Note: Do NOT store 4 M Hydroxylamine. This buffer should be freshly made on the day of use.

5.2. Protocol for purification of CyDye labeled cDNA synthesized with the Amersham CyScribe First-Strand cDNA Labeling Kit

1. Degradation of RNA

a. Add 2 µl 2.5 M NaOH (user supplied) to each labeling reaction. Mix by vortexing.

b. Spin for 30 seconds at 13 800 × g to collect contents at bottom of tube.

c. Incubate 37°C 15 minutes.

d. Add 10 µl 2 M HEPES free acid (user supplied) to each sample. Mix by vortexing.

e. Spin for 30 seconds at 13 800 × g to collect contents at bottom of tube.

f. If necessary sample can be stored at -20°C or proceed straight to step 2 below.

2. Sample Capture

a. For each purification to be performed, place one CyScribe MicroSpin column into one Collection tube.

b. Add 500 µl **Capture buffer type 1** to 20–100 µl sample.

500 µl Capture buffer type 1 to 20–100 µl sample

c. Mix thoroughly by gentle inversion. Do not vortex.

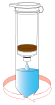
Note: Do not leave the sample in the **Capture buffer type 1** for longer than 10 minutes, as this may reduce final purified probe yield. Plan accordingly when working with large numbers of samples.

d. Load the **Capture buffer type 1**-sample mix onto the assembled CyScribe MicroSpin column and Collection tube.

Load Capture buffer type 1-sample mix

Note: the cap of the Collection tube can be used to cap the CyScribe Microspin column.

e. Spin the assembled column and Collection tube for 30 seconds at 13 800 × g.



30 seconds 13 800 × g

f. Proceed to step 3 below.

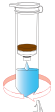
3. Wash & Dry (with Wash buffer type 3)

a. Discard flowthrough by emptying the Collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

b. Add 600 µl **Wash buffer type 3** to the column.

600 µl Wash buffer type 3

c. Spin for 30 seconds at 13 800 × g.



30 seconds 13 800 × g

d. Repeat Wash & Dry steps a–c twice for a total of 3 washes.

e. Discard the flowthrough by emptying the Collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

f. Spin for 10 seconds at 13 800 × g to dry to CyScribe MicroSpin column.

g. Proceed to step 4 below.

4. Elution (with Elution buffer type 7)

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

Note: Use of amber tubes is recommended. CyDye probes should be stored *in the dark*.

b. Add 60 µl **Elution buffer type 7** directly to the center of the membrane in the CyScribe MicroSpin column.

60 µl Elution buffer type 7

Note: Pre-warming **Elution buffer type 7** to 65°C can increase the yield by approximately 5%.

c. Incubate at room temperature (RT) for 1–5 minutes.

1–5 minutes RT

Note: Use a 5 minute incubation to achieve maximum yield.

d. Spin for 1 minute at 13 800 × g to recover the purified cDNA as flowthrough in the microcentrifuge tube.

1 minute
13 800 × g

Note: Repeating steps b.-d. can increase yield of labeled cDNA by approximately 10%, but will reduce concentration.

e. If required, determine the yield and calculate the Frequency of CyDye Incorporation as detailed in Sections 6.2 & 6.3. Store purified labeled cDNA at -20°C *in the dark*. Proceed to microarray applications.

5.3. Protocol for purification of probes synthesized with the Amersham CyScribe Post-Labeling Kit

1. Degradation of RNA (with 2.5 M NaOH & 2 M HEPES free acid)

a. Add 2 µl 2.5 M NaOH (user supplied) to each sample. Mix by vortexing.

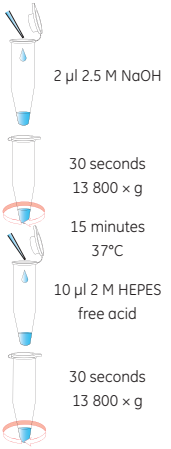
b. Spin for 30 seconds at 13 800 × g to collect contents at bottom of tube.

c. Incubate for 15 minutes at 37°C.

d. Add 10 µl 2 M HEPES free acid (user supplied) to each sample. Mix by vortexing.

e. Spin for 30 seconds at 13 800 × g to collect contents at bottom of tube.

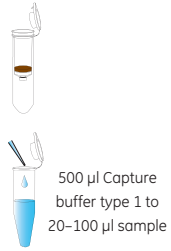
f. If necessary sample can be stored at -20°C or proceed straight to Sample capture step.



2. Sample Capture

a. For each purification to be performed, place one CyScribe MicroSpin column into a Collection tube.

b. Add 500 µl **Capture buffer type 1** to 20–100 µl sample.



Note: If using the Amersham CyScribe Post-Labeling Kit, at this point the sample volume will be 32 µl

c. Mix thoroughly by gentle inversion. Do not vortex.

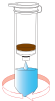
Note: Do not leave the sample in the **Capture buffer type 1** for longer than 10 minutes, as this may reduce final purified probe yield. Plan accordingly when working with large numbers of samples.

d. Load the **Capture buffer type 1**-sample mix onto the assembled CyScribe MicroSpin column and Collection tube.

Load Capture buffer type 1-sample mix

Note: the cap of the Collection tube can be used to cap the Cyscribe Microspin column.

e. Spin the assembled column and Collection tube for 30 seconds at 13 800 × g.



30 seconds
13 800 × g

f. Proceed to step 3 below.

3. Wash & Dry (with 80% Ethanol)

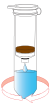
a. Discard flowthrough by emptying the Collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

b. Add 600 µl 80% Ethanol (user supplied) to the column.

600 µl
80% Ethanol

Note: Ensure that you use 80% Ethanol and not Wash buffer type 3 supplied in the kit.

c. Spin for 30 seconds at 13 800 × g.



30 seconds
13 800 × g

d. Repeat Wash & Dry steps a–c twice for a total of 3 washes.

× 3 washes

e. Discard the flowthrough by emptying the collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

10 seconds
13 800 × g

f. Centrifuge for 10 seconds at 13 800 × g to dry the CyScribe MicroSpin column.

g. Proceed to step 4 below.

4. Elution (with 0.1 M Sodium Bicarbonate pH 9.0)

a. Discard the collection tube and transfer the CyScribe MicroSpin column to a fresh DNase free 1.5 ml microcentrifuge tube (supplied by user).

b. Add 60 µl 0.1 M Sodium Bicarbonate pH 9.0 (user supplied) directly to the center of the membrane in the CyScribe MicroSpin column.

60 µl 0.1 M Sodium Bicarbonate pH 9.0

Note: Do NOT use **Elution buffer type 7** at this point, as this would result in failure of the Post-Labeling Coupling Reaction. This is due to the presence of Tris in **Elution buffer type 7**, and suboptimal pH.

Note: Pre-warming 0.1 M Sodium Bicarbonate pH 9.0 to 65°C can increase the yield by approximately 5%.

c. Incubate for 1–5 minutes at room temperature (RT).

1–5 minutes RT

Note: Use a 5 minute incubation to achieve maximum yield.

d. Spin for 1 minute at 13 800 × g to recover the purified cDNA as flowthrough in the microcentrifuge tube.

1 minute
13 800 × g

Note: Repeating steps b–d can increase yield of labeled cDNA by approximately 10%, but will reduce concentration.

e. Some users find it helpful to quantify yield at this stage using UV spectroscopy. Otherwise proceed immediately to step 5 below.

5. Post-Labeling Coupling Reaction (with 4 M Hydroxylamine & CyDye Post-Labeling Reactive Dye Pack)

Add sample to CyDye NHS ester

Note: Pulse spin the CyDye NHS ester before use to bring contents to bottom of tube. Use either Cy3 OR Cy5 NHS ester, as appropriate.

a. Add the sample directly to one aliquot of the appropriate CyDye NHS ester (user supplied). Resuspend the CyDye NHS ester completely by pipetting up and down several times.

Note: Do not vortex as this will shear large cDNA fragments.

1 minute
13 800 × g

b. Spin for 1 minute at 13 800 × g to collect sample at bottom of tube.

c. Incubate for 60–90 minutes at room temperature (RT) *in the dark*.

60–90 minute RT
in the dark

15 µl 4 M Hydroxylamine

d. Add 15 µl 4 M Hydroxylamine (user supplied) to the sample. Mix completely by pipetting up and down several times.

Note: Do not vortex as this will shear large cDNA fragments.

15 minutes RT
in the dark

e. Incubate for 15 minutes at room temperature *in the dark*.

f. Proceed immediately to step 6 below.

6. Sample Capture

a. For each purification to be performed, place one CyScribe MicroSpin column into a Collection tube.

b. Add 500 µl **Capture buffer type 1** to 20–100 µl sample.

c. Mix thoroughly by gentle inversion. Do not vortex.

Note: Do not leave the sample in the **Capture buffer type 1** for longer than 10 minutes, as this may reduce final purified probe yield. Plan accordingly when working with large numbers of samples.

d. Load the **Capture buffer type 1**-sample mix onto the assembled CyScribe MicroSpin column and Collection tube.

e. Spin the assembled column and Collection tube for 30 seconds at 13 800 × g.

f. Proceed to step 7 below.

7. Wash & Dry (with Wash buffer type 3)

a. Discard flowthrough by emptying the Collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

b. Add 600 µl **Wash buffer type 3** to the column.

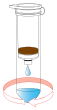
c. Spin for 30 seconds at 13 800 × g.

d. Repeat Wash & Dry steps a–c twice for a total of 3 washes.

e. Discard the flowthrough by emptying the Collection tube. Place the CyScribe MicroSpin column back inside the Collection tube.

× 3 washes

f. Centrifuge for 10 seconds at 13 800 × g to dry to CyScribe MicroSpin column.



10 seconds
13 800 × g

g. Proceed to step 8 below.

8. Elution (with Elution buffer type 7)

a. Discard the collection tube and transfer the CyScribe MicroSpin column to a fresh DNase free 1.5 ml microcentrifuge tube (supplied by user).

Note: Use of amber tubes is recommended. CyDye probes should be stored in the dark.

b. Add 60 µl **Elution buffer type 7** directly to the center of the membrane in the CyScribe MicroSpin column.

60 µl Elution buffer type 7

Note: Pre-warming **Elution buffer type 7** to 65°C can increase the yield by approximately 5%.

c. Incubate for 1–5 minutes at room temperature

1–5 minutes RT

Note: Use a 5 minute incubation to achieve maximum yield.

d. Spin 13 800 × g for 1 minute to recover the purified cDNA as flowthrough in the microcentrifuge tube.

1 minute
13 800 × g

Note: Repeating steps b.-d. can increase yield of labeled cDNA by approximately 10%, but will reduce concentration.

e. If required, determine the yield and calculate the Frequency of CyDye Incorporation as detailed in Sections 6.2 & 6.3. Proceed to microarray applications. Store purified sample 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:

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

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 3000.

6.2. Calculation of cDNA yield

The yield of cDNA purified with the illustra CyScribe GFX Purification Kit can be estimated by UV spectrophotometry at 260 nm. If the cDNA is CyDye labeled, there is only a marginal increase in absorbance values obtained (typically less than 1% contribution at the labeling densities achieved with the Amersham CyScribe First Strand cDNA Labeling Kit and the Amersham CyScribe Post-Labeling Kit.

Note: An absorbance reading of 1 at 260 nm corresponds to 37 ng/µl cDNA.

6.3. Calculation of CyDye frequency of incorporation (FOI)

The incorporation of Cy3 and Cy5 into cDNA can be quantified with spectroscopy. Using their extinction coefficients, the total amount of CyDye molecules incorporated into cDNA can be calculated. Prior purification of the labeled cDNA is essential since residual free CyDye will interfere with the measurements.

The FOI can be used as a guide to optimizing the amount of sample required as a hybridization probe. Additionally, the relative amount of

Cy3 and Cy5 in dual color hybridizations can be adjusted to account for any imbalances in the detection of these dyes with scanning instruments.

Measure the absorbance of the sample against a blank at 260 nm, and 550 nm for Cy3 or 650 nm for Cy5. Typically, 20 µl of CyDye labeled cDNA, diluted 1:5 with nuclease-free water, will give absorbance readings at 260 nm in the range of 0.01–0.1 units, when using cuvettes with a 1 cm path length.

Note: Ensure that the cuvettes are thoroughly clean before adding cDNA samples to them.

Note: The cDNA can be recovered from the measuring cuvette, dried down and used for microarray hybridizations, if necessary.

The FOI for Cy3 and Cy5 into cDNA can be calculated from their respective extinction coefficients and using the formulae below:

Extinction coefficients for Cy3 and Cy 5:
Cy3 150 000 $M^{-1}\,cm^{-1}$ at absorption maxima 550 nm

Cy5 250 000 $M^{-1}\,cm^{-1}$ at absorption maxima 650 nm

$$\text{pmoles Cydye in sample} = \frac{A}{E} \times \frac{1}{W} \times Z \times \text{dilution factor} \times 10^6$$

A = absorbance Cy3 at 550 nm or Cy5 at 650 nm
E = the extinction coefficient for Cy3 or Cy5
Z = original sample volume in microliters (µl)
W = optical path of cuvette in centimeters (cm)

$$\text{Incorporation of Cydye} = \frac{\text{pmoles Cydye in sample}}{\text{µg nucleic acid in sample}}$$

$$\text{FOI} = \text{pmoles incorporated dye} \times \frac{(324.5)}{\text{ng cDNA probe}}$$

For example, if 20 µl of Cy3-labelled cDNA at nucleic acid concentration of 0.025 µg/µl that has been diluted 5-fold gives absorbance of 0.06 at 550 nm and 0.5 cm light path, then pmoles of Cy3 in sample = 80 and incorporation of Cy3 = 160 pmoles Cy3 per µg of nucleic acid.

Note: Probes with FOI in the range of 20–50 perform well in microarray hybridizations. Probes with FOI values < 10 will generate weak signals. At FOI > 70, probes might also produce reduced signals because of quenching between CyDye fluors.

6.4. 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: DNA yield is low

Possible cause	Suggestions
Ethanol not added to Wash buffer type 3	• Add appropriate volume of Absolute Ethanol to Wash buffer type 3. Add 50 ml of Absolute Ethanol to the Wash buffer type 3 in kit 27-9606-01 or add 100 ml Absolute Ethanol to the Wash buffer type 3 in kit 27-9606-02. Mix by inversion. Indicate on the label that this step has been completed. Store upright with the cap tightly closed.
The Wash buffer type 3 and/or 80% Ethanol was not completely removed	• After the Wash & Dry centrifugation step(s), if any of the ethanolic Wash buffer comes into contact with the bottom of the column discard the flowthrough and re-centrifuge for 30 seconds. The presence of residual Ethanol may affect downstream applications and must be carefully removed.
Elution buffer type 7 and/or 0.1 M Sodium Bicarbonate pH 9.0 not added to centre of illustra CyScribe GFX MicroSpin column	• Ensure buffers used for elution are pipetted carefully onto the centre of the matrix at the bottom of the illustra CyScribe GFX MicroSpin column. Pre-warming the buffers to 65°C before use can increase yield by up to 5%.

Problem: DNA yield is low (continued)

Possible cause	Suggestions
0.1 M Sodium Bicarbonate pH 9.0 prepared incorrectly	• Follow the preparation procedure in section 5.1 for preparation of 0.1 M Sodium Bicarbonate pH 9.0. Do not elute bound probes with water. CyDye labels are positively charged and stick tightly to the membrane in the illustra CyScribe GFX MicroSpin column; always use the elution buffer recommended in the protocol.
cDNA incubated in Capture buffer type 1 for longer than 10 minutes	• Do not incubate samples in the Capture buffer type 1 for longer than 10 minutes. Plan your work carefully when working with large numbers of samples.

Problem: cDNA sample floats out a the well when loading onto gel

Possible cause	Suggestions
The Wash buffer type 3 and/or 80% Ethanol was not completely removed	• After the Wash & Dry centrifugation step(s), if any of the ethanolic Wash buffer comes into contact with the bottom of the column discard the flowthrough and re-centrifuge for 30 seconds. The presence of residual Ethanol may affect downstream applications and must be carefully removed.

Problem: cDNA sample floats out a the well when loading onto gel

Possible cause	Suggestions
Collection tube was not emptied after the centrifugation for the Sample Capture 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 Capture step.

Problem: Calculated FOI very low

Possible cause	Suggestions
Post-labeling coupling reaction was unsuccessful- incorrect Elution buffer or incorrect Wash buffer used	• Any sample used for the Post-Labeling Coupling Reaction must have been washed with 80% Ethanol and be in 0.1 M Sodium Bicarbonate pH 9.0. The Tris salt present in Wash buffer type 3 can react with CyDye NHS esters, and impact the coupling reaction. The pH of the sample should be 9 for optimal coupling, hence the requirement for 0.1 M Sodium Bicarbonate pH 9.0.

6.5. Related products

Related products available from GE Healthcare

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
Preparation of buffers	Water, nuclease-free	US70783	500 ml
Generation of Cy3 and Cy5 labeled cDNA for microarray hybridization	Amersham CyScribe First-Strand cDNA Labeling kit	RPN6200	25 reactions
	Amersham CyScribe Post-Labeling kit	RPN5660	12 × Cy3 reactions + 12 × Cy5 reactions
CyDye fluors optimized for use with samples for microarray	Cy3 and Cy5 Post-Labeling Reactive Dye Pack	RPN5661	12 × Cy3 reactions + 12 × Cy5 reactions
	Cy3 Post-Labeling Reactive Dye Pack	25-8010-79	12 × Cy3 reactions
	Cy5 Post-Labeling Reactive Dye Pack	25-8010-80	12 × Cy5 reactions

Application	Product	Product Code	Pack Size
CyDye fluors optimized for use with samples for microarray	Cy3 and HyPer5 Post-Labeling Reactive Dye Pack	28-9224-19	(12 × 40,000 pmol Cy3 plus 12 × 15,000 pmol HyPer5)
	HyPer5 Post-Labeling Reactive Dye Pack	28-9224-18	(12 × 15,000 pmol HyPer5)
Microarray	Microarray Hybridization Buffer	RPK0325	2 × 1 ml
Small scale RNA isolation High quality RNA from diverse sample types; for small amounts of precious samples	illustra RNAspin Mini Kit	25-0500-70	20 preps
High yield RNA isolation Scalable input and output	illustra RNAspin Midi Kit	25-0500-73	20 preps
Microarray controls Artificial exogenous controls	Universal Score Card	RPK3161	20 hybridizations (trial size)

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84, Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111, Freiburg,
Germany

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327,
Piscataway, NJ 08855-1327,
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For contact information for your local office,
please visit: www.gelifesciences.com/contact

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

http://www.gelifesciences.com

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The next four pages are a protocol card.
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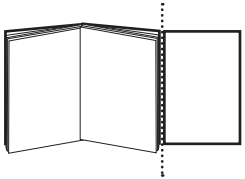


Quick Reference Protocol Card

illustra™ CyScribe™ GFX™ Purification Kit

27-9606-01 (25 purifications)
27-9606-02 (50 purifications)

A. Protocol for purification of CyDye™ labeled cDNA synthesized with the Amersham™ CyScribe First-Strand cDNA Labeling Kit

Check appropriate volume of Ethanol added to **Wash buffer type 3**

Ensure 2.5 M NaOH & 2 M HEPES free acid available

:Add :Spin :Incubate

1. Degradation of RNA (with 2.5 M NaOH & 2 M HEPES free acid)

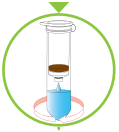
- 2 µl 2.5 M NaOH; vortex
- 30 seconds 13 800 × g
- 15 minutes 37°C
- 10 µl 2 M HEPES free acid; vortex
- 30 seconds 13 800 × g

2. Sample Capture

- Place CyScribe MicroSpin column in Collection tube
- 500 µl Capture buffer type 1 to 20–100 µl sample
- Mix by gentle inversion
- Load Capture buffer type 1 – sample mix onto CyScribe MicroSpin™ column
- 30 seconds 13 800 × g

3. Wash & Dry (with Wash buffer type 3)

- Discard flowthrough
- 600 µl Wash buffer type 3
- 30 seconds 13 800 × g
- Repeat for a total of 3 washes
- Discard flowthrough
- 10 seconds 13 800 × g



4. Elution (with Elution buffer type 7)

- Discard Collection tube. Transfer GFX MicroSpin column to a clean amber 1.5 ml DNase-free microcentrifuge tube.
- 60 µl Elution buffer type 7
- Room temperature 1–5 minutes
- 1 minute 13 800 × g
- Retain eluate
- Calculate yield and/or FOI (see sections 6.2 & 6.3) if required
- Store purified cDNA at -20°C

http://www.gelifesciences.com
GE Healthcare UK Limited.Amersham Place, Little Chalfont,Buckinghamshire, HP7 9NA UK

1–5 minutes Room temperature
1 minute 13 800 × g; retain eluate; calculate yield

5. Post-Labeling Coupling Reaction

Sample to CyDye NHS ester aliquot. Re-suspend by pipetting up and down
1 minute 13 800 × g
60–90 minutes room temperature in the dark
15 µl 4 M Hydroxylamine. Mix completely by pipetting up and down
15 minutes room temperature in the dark

6. Sample Capture

- Place CyScribe MicroSpin column in Collection tube

500 µl Capture buffer type1 to 20–100 µl sample; mix by inversion

- Load Capture buffer type 1 – sample mix onto column

30 seconds 13 800 × g

7. Wash & Dry (with Wash buffer type 3)

- Discard flowthrough

600 µl Wash buffer type 3
30 seconds 13 800 × g

- Repeat for a total of 3 washes; discard flowthrough

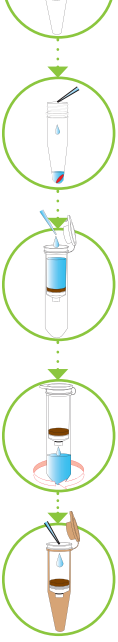
10 seconds 13 800 × g

8. Elution (with Elution buffer type 7)

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

60 µl Elution buffer type 7
1–5 minutes room temperature
1 minute 13 800 × g; retain eluate; calculate yield and/or FOI

- Store purified sample at -20°C



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27-9606-01 PC AF 06 2009

Quick Reference Protocol Card

illustra CyScribe GFX Purification Kit

27-9606-01 (25 purifications)
27-9606-02 (50 purifications)

B. Protocol for purification of probes synthesized with the Amersham™ CyScribe Post-Labeling Kit

• Check appropriate volume of Ethanol added to Wash buffer type 3 • Ensure 2.5 M NaOH, 2 M HEPES free acid, 80% Ethanol, 0.1 M Sodium Bicarbonate pH 9.0 & CyDye Post-Labeling Reactive Dye Pack available

:Add :Spin :Incubate

1. Degradation of RNA (with 2.5 M NaOH & 2 M HEPES free acid)

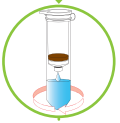
- 2 µl 2.5 M NaOH; vortex
- 30 seconds 13 800 × g
- 15 minutes 37°C
- 10 µl 2 M HEPES free acid; vortex
- 30 seconds 13 800 × g

2. Sample Capture

- Place column in Collection tube
- 500 µl Capture buffer type 1 to 20–100 µl sample; mix by inversion
- Load Capture buffer type 1 – sample mix onto column
- 30 seconds 13 800 × g

3. Wash & Dry (with 80% Ethanol)

- Discard flowthrough
- 600 µl 80% Ethanol
- 30 seconds 13 800 × g
- Repeat for a total of 3 washes; discard flowthrough
- 10 seconds 13 800 × g



4. Elution (with 0.1 M Sodium Bicarbonate pH 9.0)

- Discard Collection tube. Transfer GFX MicroSpin column to a clean 1.5 ml DNase-free microcentrifuge tube.
- 60 µl 0.1 M Sodium Bicarbonate pH 9.0