GE Healthcare

Amersham CyScribe Array CGH Genomic DNA Labeling System

Product Booklet

Code: 28-9199-56 (30 reactions)

Page Finder

1. Legal

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http//www.gehealthcare.com/lifesciences

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. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

The CyScribe™ Array CGH Labeling Kit is shipped in dry ice and should be stored between -15°C and -30°C. The illustra™ GFX™ PCR DNA and Gel Band Purification Kit (28903470) is shipped at ambient temperature and should be stored at ambient temperature.

2.3. Expiry

See outer packaging for expiry details.

3. Components

3.1. System components

1.) LABELING MODULE

CyScribe Array CGH Genomic DNA Labeling Kit

Component	Total Volume	Number of vials
1. Random nonamer	165 µl	2
2. Reaction buffer	300 µl	1
3. dCTP-labeling mix	240 µl	1
4. Nuclease free water	2000 µl	2
5. Cy3-dCTP (1 nmol/µl)	50 µl	2
6. Cy5-dCTP (1 nmol/µl)	50 µl	2
7. Klenow (Exo Free)	30 µl	1
8. 0.5 M EDTA solution	150 µl	1
9. Lambda control DNA	100 µl	1

2.) PURIFICATION MODULE

illustra GFX PCR DNA and Gel Band Purification Kit (28903470)

3.2. Reagents and equipment to be supplied by the user

In addition to the CyScribe Array CGH Genomic DNA Labeling System components, you need the following reagents and equipment to complete the labeling reactions:

- Water—Use only deionized or distilled water that is sterile and free of contaminating nucleic acid.
- Genomic DNA
- 100% ethanol
- Gloves
- Vortex mixer
- Incubators
- Ice bucket with ice
- 0.5 ml and 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Sterile pipette tips
- Water baths set at 95ºC / 37ºC / 70ºC or a thermal cycler

4. Description

Chromosomal aberrations (deletions, gains and amplifications) are hallmarks of several cancers. Identification of the genes affected in these regions could lead to the development of markers for prognosis and targets of therapy. Conventional Comparative Genomic Hybridization (CGH) is in practice in cytogenetics laboratories to identify chromosomal abnormalities. Array CGH or matrix based CGH is a microarray based method for analyzing a whole genome in a single experiment to detect copy number changes. Normal and disease genomic DNA samples are labeled differentially using different fluorophores and competitively hybridized on to the glass slide pre-arrayed with normal DNA (oligonucleotide or genomic DNA fragments). The fluorescent intensities for each spot on the slide are measured and ratios of intensities for both fluorophores are calculated to obtain the difference in gene copy number between samples.

The CyScribe Array CGH Genomic DNA Labeling System allows genomic DNA from various sources to be labeled *in vitro* to high specific activity with Cy™3 and Cy5 labeled dCTP using a mutant form of the Klenow fragment of DNA polymerase I [Klenow (Exo Free)], random nonamers, and optimized dCTP-labeling mix. This kit includes a labeling module with all the reagents for 30 labeling reactions and a purification module to purify the labeled DNA probes.

5. Protocol

5.1. Preliminary preparations and general handling instructions

Please follow these precautions when preparing labeling reactions and handling dye-labeled nucleotides:

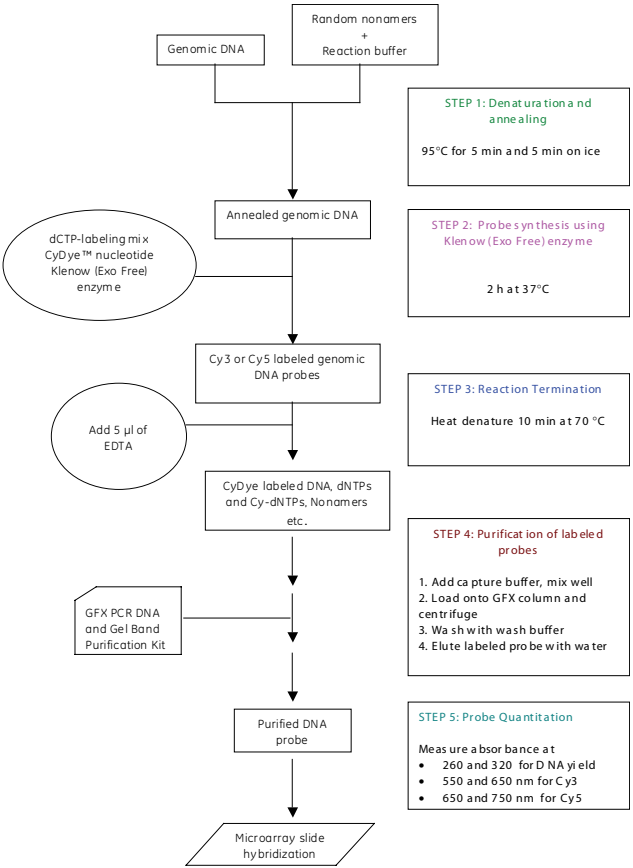
1. **Wear gloves at all times:** Nucleases and phosphatases from unguarded fingers degrade nucleotides and DNA probes. Powder and talc from some brands of gloves will bind nucleic acids and are also highly fluorescent.
2. **Store dye-labeled nucleotides at –20ºC in the dark:** Aliquot all dye-labeled dNTPs into single-use portions to minimize freeze-thaw cycles. Nucleotide solutions are stable for many years if kept frozen at -20°C. The phosphate bonds are progressively broken when subjected to repeated rounds of freezing and thawing.
3. **Protect dye-labeled dNTPs from light at all times:** All exposures to light must be minimal and restricted to the time taken to complete a required operation. Perform experimental procedures in low light conditions if possible.
4. **Treat labeled probes with the same care as dye-labeled dNTPs:** Store labeled probes inside a lightproof container to protect from refrigerator lights. dNTPs are stable for up to 3 months at 4°C in the absence of divalent cations and at a slightly alkaline pH of 8.
5. **Thaw all components prior to use:** Thaw all components of the kit completely on ice and mix contents before dispensing from the vials.

5.2. Isolation of genomic DNA

Genomic DNA can be isolated using the user's method of choice. The illustra Tissue and Cells Genomic Prep Mini (28904275) and Midi (28904273) Kits or the illustra Blood Genomic Prep Mini (28904264) and Midi (28904261) Kits provide complete reagents for isolating genomic DNA of sufficient quality and quantity for this method.

If the genomic DNA is available in limited quantities, we recommend using the illustra GenomiPhi™ HY DNA Amplification Kit (25660020) or the illustra GenomiPhi V2 DNA Amplification Kit (25660030) to obtain suitable quantities of DNA required for labeling reactions.

5.3. Outline of the labeling protocol



5.4. Genomic DNA probe labeling reactions

1. Add the following reaction components sequentially to a 0.5 ml centrifuge tube on ice:

Component	Volume	
	Cy3 labeling reactions	**Cy5 labeling reactions**
Genomic DNA 1 µg*	-- µl	-- µl
Random nonamers	10 µl	10 µl
Reaction buffer	10 µl	10 µl
Nuclease free water	-- µl	-- µl
Total Volume (Adjust by adding Nuclease free water supplied with the kit)	**38 µl**	**38 µl**

Notes: *Recommended per reaction

2. Mix the reaction components by pipetting gently.

3. Incubate the reaction mixture at 95°C for 5 minutes in a water bath or thermal cycler then cool on ice for 5 minutes.

4. Centrifuge the tubes briefly to collect all reaction components at the bottom of the tube and place on ice. Add the following labeling components sequentially to the 38 µl sample:

Component	Volume	
	Cy3 labeling reactions	Cy5 labeling reactions
dCTP-labeling mix	8 µl	8 µl
Cy3- or Cy5-dCTP	3 µl	3 µl
Klenow (Exo Free)	1 µl	1 µl
Total Volume	**50 µl**	**50 µl**

Note: A control reaction with all components except enzyme is recommended for every experiment. The absorbance values obtained from this reaction are used for background subtraction during probe quantification if needed.

5. Mix reactions by pipetting gently and spin briefly to collect the components at the bottom of the tubes.

6. Incubate reactions in a thermal cycler or water bath at 37°C for 2 hours.

5.5. Reaction termination

7. Add 5 µl of 0.5 M EDTA (pH 8.0) to each 50 µl reaction tube and incubate at 70°C for 10 minutes. Cool on ice for 5 minutes.

5.6. Purification of genomic DNA probes

For efficient probe purification and minimal background during hybridization, use the optimized glass fiber filter illustra GFX PCR DNA and Gel Band Purification Kit (supplied) as recommended.

The wash buffer requires dilution before use. Add 48 ml of absolute ethanol to the bottle containing the wash buffer. Mix by inversion.

1. Add 500 µl of capture buffer to each reaction, mix thoroughly by pipetting up and down 4-6 times, and transfer entire solution to one GFX column placed in a collection tube.
2. Filter samples by centrifugation at full speed (13 000 rpm) for 30 seconds. Discard the flow-through. Place the GFX column back inside the collection tube.
3. Add 500 µl of wash buffer to the column. Centrifuge at full speed (13 000 rpm) for 30 seconds.
4. Discard the collection tube and transfer the GFX column to a fresh 1.5 ml microcentrifuge tube (i.e. NOT a collection tube).
5. Apply 50 µl of autoclaved double-distilled water directly to the top and center of the glass fiber matrix in the GFX column. Incubate the sample in the dark at ambient temperature for 1 minute.
6. Centrifuge at full speed (13 000 rpm) for 1 minute to recover the purified probe.
7. Store the labeled probe in the dark at -20°C or proceed to probe quantification.

5.7. Quantification of labeled DNA probes

Purify the labeled DNA probes according to the protocol in Section 5.6. prior to quantification of the DNA probe. Any residual unincorporated labeled nucleotides will interfere with the detection of the labeled DNA probe.

Genomic DNA probes are quantitated by UV/visible spectrometry by measuring absorbance at 260, 320, 550 and 650 nm for Cy3 labeled probes and at 260, 320, 650 and 750 nm for Cy5 labeled probes. Use a quartz microcuvette for quantitation. We recommend measuring at least 1:1 ratio diluted probes. However, probes may be diluted up to a 1:10 ratio for quantitation.

a) DNA yield:

DNA {ng} = {A_{260}-A_{320}} × 50 × elution volume {50 µl}

The standard genomic labeling reactions will produce DNA yields of 4.5–6.0 µg.

b) Amount of fluorescently labeled dye:

Cy3 {pmole} = {A_{550}-A_{650}} / 0.15 × elution volume {50 µl}

Cy5 {pmole} = {A_{650}-A_{750}} / 0.25 × elution volume {50 µl}

The standard genomic labeling reactions will produce 250–350 pmoles for Cy3 labeling reactions and 200–300 pmoles for Cy5 labeling reactions.

c) Nucleotide/Dye ratio:

Cy3 = {A_{260} × 150 000 [cm^{-1} M^{-1}]} / {A_{550} × 6600}

Cy5 = {A_{260} × 250 000 [cm^{-1} M^{-1}]} / {A_{650} × 6600}

The standard genomic labeling reactions will produce nucleotide to dye ratio values between 50-65 for Cy3 reactions and 60–75 for Cy5 reactions.

5.8. Hybridization protocol

CyDyes are a range of fluorescent dyes based on the benefits of Cyanine fluors. Cy3 and Cy5 offer bright and intense colors with narrow emission spectra that make them ideal for multicolor detection in microarray and other fluorescence based genome analysis techniques. Key benefits from the use of Cy3- and Cy5-labeled DNA are their high sensitivity, relatively high photostability, insensitivity to pH and high water solubility. We recommend that the users of the CyScribe Array CGH Genomic DNA Labeling System follow the instructions provided with their microarray slides and analysis systems for specific information about using those reagents and equipment in microarray analysis.

6. Use of Lambda control DNA

The CyScribe Array CGH Genomic DNA Labeling System contains *Enterobacteria phage* λ genomic DNA for control labeling reactions. This DNA can be used as a template for labeling reactions using the standard protocol described in Section 5.

Figure 1 shows a comparison of the DNA yield typically obtained using the Lambda control DNA supplied in the kit and human genomic DNA, and Figure 2 shows a comparison of the dye incorporation typically obtained using the Lambda control DNA supplied in the kit and human genomic DNA.

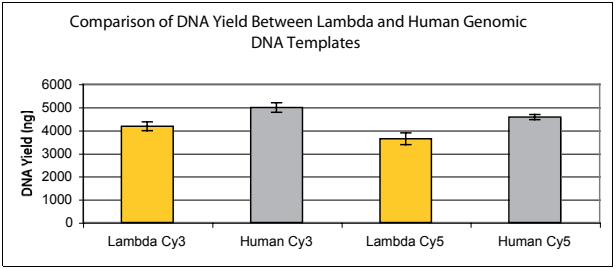


Fig 1. A DNA yield comparison using 1 µg of either Lambda control DNA template or human genomic DNA template following the CyScribe Array CGH Genomic DNA Labeling System standard protocol.

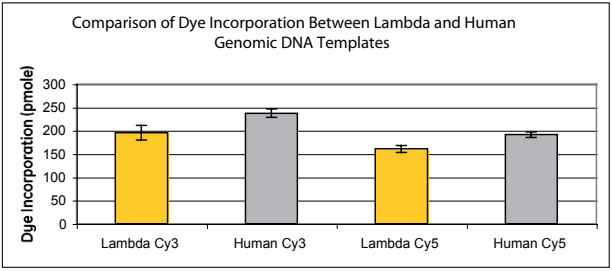


Fig 2. A dye incorporation comparison using 1 µg of either Lambda control DNA template or human genomic DNA template following the CyScribe Array CGH Genomic DNA Labeling Kit standard protocol.

7. Troubleshooting guide

All batches of the CyScribe Array CGH Genomic DNA Labeling System are the subject of careful quality control before shipping to ensure satisfactory performance. Should poor results be obtained we recommend the following points for consideration.

- Ensure that the procedures have been followed as specified in the standard protocol.
- Perform labeling reactions with the Lambda control DNA provided in the kit according to the protocol detailed in Section 6 "Use of Lambda control DNA". Monitor the yield of the labeled genomic DNA probes and the incorporation of CyDye according to the protocols detailed in Section 5.7. "Quantification of labeled DNA probes".
- Check the purity and concentration of the genomic DNA. For efficient labeled DNA probe synthesis, it is important that the genomic DNA template be free of contaminating proteins and RNA. It is also important that the DNA is accurately quantified before use in labeling reactions.
- If poor results are obtained in a microarray application using probes prepared with the CyScribe Array CGH Genomic DNA Labeling System, consider each of the steps of the whole microarray procedure in troubleshooting. The microarray signal observed from the microarray slide reflects the success of all the preceding steps, and failure in any of these steps can cause poor results. It is recommended to always check the success of the labeling step and to quantify the purified labeled probes before setting up microarray hybridization reactions.

Problem	Possible cause	Remedy
1. Low signal on microarray	Incomplete denaturation of template DNA.	Ensure that denaturation protocol is followed.
	Low probe concentration.	Accurately measure the concentration of template DNA used in the labeling reactions.
		Check recovery of probe if purification is performed to remove unincorporated nucleotide.
2. Little or no probe synthesized	One or more of the reaction components is missing or wrong components used	Repeat labeling reactions using correct reagents and volumes recommended at every step of the protocol.
	The amounts of CyDye nucleotide and nucleotide mix were not pipetted accurately.	Check the accuracy of pipettes and repeat labeling reaction with correct amounts.
	The amount of DNA template was less than recommended.	Re-analyze the concentration of DNA template or use more DNA in the labeling reaction.

Problem	Possible cause	Remedy
3. Low recovery of labeled DNA probe	Samples have been excessively exposed to light during probe synthesis, purification, or quantification.	Repeat analysis and minimize exposure of samples to light at all times.
	The ethanol concentration in the wash buffer was less than required.	Add absolute ethanol to wash buffer prior to use and cap bottle tightly for storage.
	Poor elution of fragments from GFX column.	Elution liquid must cover all of the capture membrane. Place water in the middle of the column.

8. Related products

CyDye fluors are available as CyDye deoxynucleotides, CyDye ribonucleotides, highly reactive NHS esters, Cy Direct™ Labeling reagents and CyScribe microarray labeling kits.

CyDye nucleotides

Cy3-dCTP	25 nmol	PA53021
Cy5-dCTP	25 nmol	PA55021
Cy3-dUTP	25 nmol	PA53022
Cy5-dUTP	25 nmol	PA55022
Cy3.5-dCTP	25 nmol	PA53521
Cy5.5-dCTP	25 nmol	PA55521
Cy3-dUTP	100 nmol	PA53026
Cy5-dUTP	100 nmol	PA55026

CyDye value packs

Cy3-dCTP 25 nmol × 5 plus Cy5-dCTP 25 nmol × 5	PA53031
Cy3-dUTP 25 nmol × 5 plus Cy5-dUTP 25 nmol × 5	PA55031

CyScribe First-Strand cDNA Labeling Kits

CyScribe First-Strand cDNA Labeling Kit	25 reactions	RPN6200
CyScribe First-Strand cDNA Labeling Kit with CyScribe GFX Purification Kit	25 reactions	RPN6200X
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3-dUTP and 25 nmol Cy5-dUTP	50 reactions	RPN6201
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3-dUTP and 25 nmol Cy5-dUTP with CyScribe GFX Purification Kit	50 reactions	RPN6201X

CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3-dCTP and 25 nmol Cy5-dCTP	50 reactions	RPN6202
CyScribe First-Strand cDNA Labeling System with 25 nmol Cy3-dCTP and 25 nmol Cy5-dCTP with CyScribe GFX Purification Kit	50 reactions	RPN6202X

CyScribe Post-Labeling Kits

CyScribe Post-Labeling Kit 12 vials of Cy3 and 12 of Cy5	24 reactions	RPN5660
CyScribe Post-Labeling Kit 12 vials of Cy3 and 12 of Cy5 with CyScribe GFX Purification Kit	24 reactions	RPN5660X
CyScribe Post-Labeling Reactive Dye Pack 12 vials of Cy3 and 12 of Cy5	24 reactions	RPN5661
CyScribe Direct™ mRNA Labeling Kit 12 vials of Cy3 and 12 of Cy5	24 reactions	RPN5665

illustra™ purification kits

illustra Tissue and Cells Genomic Prep Mini Spin Kit	50 purifications	28904275
illustra Tissue and Cells Genomic Prep Mini Spin Kit	250 purifications	28904276
illustra Tissue and Cells Genomic Prep Midi Spin Kit	25 purifications	28904273
illustra Blood Genomic Prep Mini Spin Kit	50 purifications	28904264
illustra Blood Genomic Prep Mini Spin Kit	250 purifications	28904265

illustra Blood Genomic Prep Midi Spin Kit	25 purifications	28904261
illustra Blood Genomic Prep Midi Spin Kit	100 purifications	28904262
illustra CyScribe GFX Purification Kit	25 reactions	27960601
illustra CyScribe GFX Purification Kit	50 reactions	27960602

illustra GenomiPhi DNA amplification kits

illustra GenomiPhi HY DNA Amplification Kit	100 reactions	25660020
illustra GenomiPhi HY DNA Amplification Kit	1000 reactions	25660025
illustra GenomiPhi V2 DNA Amplification Kit	25 reactions	25660030
illustra GenomiPhi V2 DNA Amplification Kit	100 reactions	25660031
illustra GenomiPhi V2 DNA Amplification Kit	500 reactions	25660032

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 UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

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

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

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