Product Manual

CGH Labeling Kit for BAC Arrays

Catalog #: ENZ-42670

20 labeling reactions

- **for preparation of Cyanine 3- and Cyanine 5-labeled DNA**
- **for hybridization to BAC arrays**

Note:
20 reaction size kit contains reagents sufficient for 20 reactions or 10 reactions in duplicate with a dye swap.

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTRODUCTION

Enzo Life Sciences' **CGH Labeling Kit for BAC Arrays** (Cat. No. ENZ-42670) has been optimized to label DNA for dual sample comparative genomic hybridization (CGH) assays on BAC arrays. All required reagents are provided including fully optimized nucleotide mixes that contain either cyanine 3-dUTP or cyanine 5-dUTP.

The Enzo **CGH Labeling Kit for BAC Arrays** has been specifically tailored to meet the varying requirements of individual laboratories by providing sufficient amount of reagents for two different protocols. For the average user, the Standard Protocol calls for a 4-hour labeling in a 50µl reaction volume. For users with dilute DNA (≤ 20ng/µl), the Dilute DNA Protocol calls for a 4-16 hour labeling in a total volume of 100µl.

REAGENTS PROVIDED AND STORAGE

The **CGH Labeling Kit for BAC Arrays** is shipped on dry ice.

Upon receipt, store all reagents at –20°C in a nonfrost-free freezer. Avoid repeated freezing and thawing of the cyanine-labeled deoxynucleotides and the enzymes.

Cyanine 3-dUTP Nucleotide Mix (Vial 2) and Cyanine 5-dUTP Nucleotide Mix (Vial 3) are light sensitive. Protect from light exposure at all times.

The product is stable until the expiration date indicated on the box when stored properly.

The following reagents are provided in the kit and are sufficient for 2 x 10 labeling reactions.

Reagent	Min. Volume Supplied	Vial ID
Primers/Reaction Buffer	2 x 400µl	**1**
Cyanine 3-dUTP Nucleotide Mix	2 x 50µl	**2**
Cyanine 5-dUTP Nucleotide Mix	2 x 50µl	**3**
Klenow DNA Polymerase	1 x 20µl	**4**
Stop Buffer	1 x 100µl	**5**
Nuclease-free Water	1 x 1ml	**W**

OTHER MATERIALS NEEDED

1. Water bath, heating block,or incubator set at 37°C and 99°C or a PCR machine with heated-lid
2. DNA purification columns (QIAquick PCR Purification Kit, Qiagen No. 28104)
3. *Optional*: *Dpn II* restriction enzyme and buffer
4. *Optional*: Sonicator
5. *Optional*: TE buffer (10mM Tris, 1mM EDTA, pH 8.0)
6. *Optional:* Additional water bath, heating block,or incubator set at 65°C or a PCR machine with heated-lid

SAFETY WARNINGS AND PRECAUTIONS

- This product is for research use only. It is not intended for diagnosis of diseases in humans or animals. DO NOT use internally or externally in humans or animals.
- To avoid photobleaching of cyanine-labeled nucleotides, perform all manipulations in amber microcentrifuge tubes or protected from light by other means.
- The Klenow Exo- DNA Polymerase contains dithiothreitol (DTT). DTT causes irritation to the skin, eyes and respiratory tract. It is harmful if swallowed or inhaled. Should the solution come in contact with skin or eyes, wash immediately with water.

METHODS AND PROCEDURES

AMOUNT OF DNA REQUIRED

- Enzo's **CGH Labeling Kit for BAC Arrays** is optimized for use with 0.15–2.0µg genomic DNA as starting material for BAC arrays.
- In general, 0.5–1µg of input genomic DNA is sufficient for labeling and hybridization to most BAC array formats. Lower amounts of input DNA (0.15–0.5µg) should be sufficient with small hybridization volume format arrays, long hybridization times (60 hours) or active mixing hybridization chambers.
- We recommend using higher amounts of input DNA (0.5–2.0µg) when performing CGH analysis on genomic DNA isolated from **FFPE** (formalin-fixed paraffin embedded) tissues. Under conditions where input genomic DNA is limiting (< 200 ng) and/or isolated from FFPE tissues, we recommend the use of Enzo's ***BioScore*™ Screening and Amplification**

Kit (Cat. No. ENZ-42440). The ***BioScore*™ Screening and Amplification Kit** is an isothermal whole genome amplification method that generates greater than 15µg of DNA from 100 ng of high quality genomic DNA in one hour. FFPE DNA samples suitable for CGH array analysis are identified by high yields in the amplification reaction. DNA samples(0.5–2.0µg) amplified by Enzo's ***BioScore*™ Screening and Amplification Kit** can then be directly labeled for array CGH analysis without restriction digestion. Both sample and reference genomic DNAs should be subjected to amplification before labeling.

ISOLATING GENOMIC DNA

- Isolate genomic DNA using an established protocol or commercially available kit.
- Determine concentration and purity of the genomic DNA by measuring the absorbance at 260nm and 280nm. We recommend the use of the NanoDrop ND-1000 UV-VIS Spectrophotometer or equivalent. The A_{260}/A_{280} ratio should be approximately 1.8 and A_{260}/A_{230} ratio should be 2.0 or greater. Significant deviation from these ratios suggests the presence of contaminants and indicates that the sample should be repurified.
- High quality, high molecular weight genomic DNA can be used directly for cyanine labeling and array hybridization without restriction digestion.

DIGESTING AND PURIFYING THE GENOMIC DNA (OPTIONAL)

- Depending upon the type of BAC array and its manufacturer, the genomic DNA may be subjected to digestion or fragmentation to reduce the size before labeling. Neither restriction digestion nor fragmentation is necessary when using the **CGH Labeling Kit for BAC Arrays**, regardless of the array format
- Genomic DNA isolated from FFPE tissues and reference control DNA should not be digested.
- When digestion is performed, both the sample and reference control DNAs should be digested with a restriction enzyme such as *Dpn II*, to produce genomic DNA fragments averaging ≤ 5 kb in length as determined by agarose gel electrophoresis. Alternatively, both the sample and reference control DNAs can be sonicated to a specified size distribution.
- To purify/concentrate the digested or fragmented genomic DNA, we recommend the use of Qiagen's QIAquick

purification columns (Qiagen No. 28104). Do not load more than 10µg DNA per column.

- Determine the concentration and purity of the digested DNA by measuring the absorbance at 260nm and 280nm. We recommend the use of the NanoDrop ND-1000 UV-VIS Spectrophotometer or equivalent.

LABELING THE DNA

Labeled DNA is prepared by the incorporation of cyanine dyelabeled nucleotides according to one of two protocols. The Standard Protocol (50µl, 4-hour labeling reaction) is described below and summarized in the table that follows (Standard Procedure Overview). The Dilute DNA Protocol (100µl, 16-hour labeling reaction) is also described (see page 6) and is summarized in the Dilute DNA Procedure Overview (see table on page 7). This protocol is specifically tailored to accommodate dilute genomic DNA solutions (≤ 20ng/ml) or where overnight labeling is preferred.

For each pair of genomic DNAs to be compared, label one sample with cyanine 3 and the other with cyanine 5. If further validation is desired, the labels can be swapped in a parallel or subsequent experiment.

STANDARD PROTOCOL (50µl, 4-Hour Labeling Reaction)

1. **Denature DNA and Anneal Random Primers**.
   a. Combine 0.15-2.0µg of genomic DNA or 0.5–2.0µg of BioScore-amplified DNA (up to 19µl) with 20µl of Primers/Reaction Buffer (Vial 1), and add enough Nuclease-free Water (Vial W) to bring the reaction mixture to 39µl.
   b. Heat at 99°C for 10 minutes and place on ice for 5 minutes. Briefly centrifuge, and return to ice.
2. **Extend Primers with Klenow Exo- DNA Polymerase**.
   a. While on ice, add 10µl of the appropriate cyanine dye-labeled nucleotide mix (Vial 2 or 3) and 1µl of Klenow Exo- DNA Polymerase (Vial 4) to the primer annealed DNA sample. Flick tube gently to mix contents. Briefly centrifuge.
   b. Incubate the reaction at 37°C for 4 hours.
   c. Add 5µl of Stop Buffer (Vial 5), mix and briefly centrifuge.

   NOTE: If purifying the labeled DNA, heat the tube for 10 min at 65°C to heat-inactivate the enzyme, cool to room temperature and centrifuge briefly.

STANDARD PROCEDURE OVERVIEW

Step	Component/Condition	Amount
1. Add	DNA (0.15–2.0µg)	up to 19µl
2. Add	Primers/Reaction Buffer (Vial 1)	20µl
3. Add	Water (Vial W)	to 39µl
4. Incubate	99°C , 10 min	
5. Incubate	Ice, 5 min	
6. Add	Nucleotide Mix (Vial 2 or 3)	10µl
7. Add	Klenow Exo- DNA Polymerase (Vial 4)	1µl
8. Incubate	37°C, 4 hours	
9. Add	Stop Buffer (Vial 5)	5µl

DILUTE DNA PROTOCOL (100µl, 4-16 Hour Labeling Reaction)

1. **Denature DNA and Anneal Random Primers.**
   a. Combine 0.15-2.0µg of genomic DNA or 0.5–2.0µg of BioScore-amplified DNA (up to 49µl) with 40µl of Primers/Reaction Buffer (Vial 1), and add enough Nuclease-free Water (Vial W) to bring the reaction mixture to 89µl.
   b. Heat at 99°C for 10 minutes and place on ice for 5 minutes. Briefly centrifuge, and return to ice.
2. **Extend Primers with Klenow Exo- DNA Polymerase.**
   a. While on ice, add 10µl of the appropriate cyanine dye-labeled nucleotide mix (Vial 2 or 3) and 1µl of Klenow Exo- DNA Polymerase (Vial 4) to the primer annealed DNA sample. Flick tube gently to mix contents. Briefly centrifuge.
   b. Incubate the reaction at 37°C for 4-16 hours depending upon work flow or preference.
   c. Add 5µl of Stop Buffer (Vial 5), mix and briefly centrifuge.

   NOTE: If purifying, heat the tube for 10 min at 65°C to heat-inactivate the enzyme, cool to room temperature and centrifuge briefly.

DILUTE DNA PROCEDURE OVERVIEW

Step	Component/Condition	Amount
1. Add	DNA (0.15–2.0µg)	up to 49µl
2. Add	Primers/Reaction Buffer (Vial 1)	40µl
3. Add	Water (Vial W)	to 89µl
4. Incubate	99°C, 10 min	
5. Incubate	Ice, 5 min	
6. Add	Nucleotide Mix (Vial 2 or 3)	10µl
7. Add	Klenow Exo-DNA Polymerase (Vial 4)	1µl
8. Incubate	37°C, 4-16 hours	
9. Add	Stop Buffer (Vial 5)	5µl

PURIFYING AND CHARACTERIZING THE LABELED DNA

1. **Purify the Labeled DNA**
   a. Purify each labeling reaction separately.
   b. Qiagen's QIAquick purification columns are recommended in this step (follow manufacturer's protocol).
   c. Elute twice with 25µl Buffer EB (supplied in Qiagen's kit) for a total of 50µl.
2. **Determine the Yield and Incorporation**

   We recommend using a NanoDrop ND-1000 UV-VIS Spectrophotometer in the Microarray Measurement Mode to determine yield and dye incorporation. Use 1.5µl from each sample to measure.

   For a typical labeling reaction, with an input of 850 ng of high quality genomic DNA, the expected yield should be at least 4µg. This DNA should contain either at least 200 pmols of incorporated Cyanine 3 or at least 130 pmols of incorporated Cyanine 5.

PREPARING THE LABELED DNA FOR ARRAY HYBRIDIZATION

Follow the array manufacturer's recommendations for preparing the labeled DNA for hybridization. The combined volume of both labeling reactions (~100µl), however, is too large for most array hybridization formats. The labeled DNA needs to be concentrated in the absence or presence of carrier DNA and COT-1 DNA. An established protocol that has shown to perform consistently well for several different array platforms is presented below.

1. Combine the cyanine 3- and cyanine 5-labeled DNA eluates.
2. Add yeast tRNA (500µg) and COT-1 DNA (100µg) to the combined DNA eluates.
3. Precipitate the DNA by the addition of 0.1 volume of 3M sodium acetate (pH 5-8) followed by 2.5 vol of ice-cold ethanol. After 10 minutes at -70° C, pellet the precipitated DNA by centrifugation for 10 minutes. Remove the supernatant carefully and wash the pellet once with 70% ethanol. Re-centrifuge for 5 minutes and remove the supernatant. Air-dry the pellet in the dark.
4. Dissolve the labeled DNA in the appropriate hybridization buffer and blocking reagents specific for the BAC arrays being used.
5. Perform pre-hybridization, hybridization, array washing and scanning as specified by the array manufacturer.

TROUBLESHOOTING

1. **Low yield and incorporation**. Poor labeling often reflects low quality input DNA. This can be due to the presence of contaminants from the purification of genomic DNA and/or the presence of degraded DNA and/or cross-linked DNA isolated from FFPE tissues.
2. **Poor signal to noise ratio despite good incorporation**. Low signal can be caused by inappropriate hybridization conditions (too stringent buffer or excessive temperature) or when the hybridization time is too short. High background can be caused by inadequate blocking and/or washing conditions. Excessive amounts of labeled DNA can also result in high background.
3. **Bright yellow array with reduced magnitude of expected changes**. This can be due to insufficient COT-1 DNA, blocking agents or hybridization and washing conditions that are not stringent enough.

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