User Guide

Version 1.2 (February 2009)

Product: **ULS™ arrayCGH Labeling Kit**
Catalog number: EA-005, EA-005A and EA-005B
Lot number: See label on package
Unit Size: 10 dual color labeling reactions or 20 single color labeling reactions
Storage conditions: See below

For the full manual which includes troubleshooting, DoL calculation in detail and the principle of ULS labeling please visit www.kreatech.com

A. Components & Storage – IMPORTANT

Component	Amount	Storage
Cy™3-ULS	10 µL or 20 µL (EA-005A)	2-8℃
Cy™5-ULS	10 µL or 20 µL (EA-005B)	2-8℃
10 x Labeling Solution	100 µL	2-8℃
KREA*pure*™ columns	20 pcs	2-8℃
KREA*block*™	1 mL	–20℃*
KREAHYB-CGH	2 mL	2-8℃

***KREA*block* is shipped at 2-8℃, store immediately at -20℃ upo n arrival.**

Reagents not included in kit

The following reagents are not supplied but may be required. All reagents should be of molecular biology grade and free of nucleases. Reference DNA, Fragmentation reagents, Ammonium Acetate (10 M), Ethanol (96-100%), Human C_0t-1 DNA (product EA-020), Yeast tRNA (Invitrogen, # 15401-029).

B. General Information

I. Background

With the advent of BAC microarrays, an unprecedented high resolution (< 1 Mb) for whole genome scanning within one single experiment has now become feasible. Array-based Comparative Genomic Hybridization (array CGH) onto microarrays is rapidly becoming the method of choice for genome wide DNA copy number screening.

II. The ULS arrayCGH Labeling Kit

The ULS array CGH Labeling Kit offers a novel non-enzymatic protocol that allows direct labeling of unmodified genomic DNA. This protocol has been designed and developed to provide a quick and highly reproducible labeling for arrayCGH analysis on microarrays. The ULS arrayCGH Labeling Kit provides enough reagents for 10 dual or 20 single hybridizations of 2 µg of genomic DNA per color.

C. Protocol

I. Genomic DNA Isolation

For Genomic DNA isolation we recommend the use of the QIAGEN QIAmp kit. The DNA material should be RNase treated as described by QIAGEN. For all DNA $OD_{260/280}$ should be >1.8 and $OD_{260/230}$ > 1.8.
Note: Be aware that some components in silica based purification systems can inhibit the ULS reaction. A final wash step using 80% ethanol (PA) before elution will prevent this. Alternatives: alcohol precipitation of eluted material or cleanup using an EDTA, Sephadex combination.

II. DNA Amplification (optional)

If the amount of isolated DNA is insufficient the sample may be amplified using commercial available kits.

III. DNA Fragmentation

When using fresh genomic DNA as sample, fragmentation is always necessary. If you are using DNA isolated from FFPE samples, fragmentation might be needed depending on fragment sizes. Optimal fragment size for array CGH applications is 200-600bp.
Fragment your DNA according to your own protocol. We recommend using heat fragmentation or sonication (see www.kreatech.com for information on protocols).
Note: In order to obtain optimal labeling densities, it is very important to determine the DNA concentration before starting the labeling procedure.

IV. DNA Labeling

a. ULS Labeling Procedure

Ratio of ULS per microgram target: 1µL of ULS-dye to exactly 2 µg DNA. This reaction can be scaled up but this ratio must remain the same.
(Example for labeling 2µg DNA)

1. Add 1µL of Cy3-ULS or Cy5-ULS to exactly 2µg DNA
2. Adjust with ultrapure water to final volume (preferably end volume of the labeling mixture is 20µL; see example)
3. Label sample by incubation for 30 minutes at 85ºC
4. Labeled samples can be stored on ice until dye removal using the KREA*pure* columns

Example of -ULS labeling of 2 µg DNA:

DNA (2 µg)	17.0 µL
Cy-ULS	1.0 µL
10 x Labeling Solution	2.0 µL
Total volume	20.0 µL

b. Dye Removal Using KREA*pure* Columns

(20800 g is equivalent to 14,000 rpm on eppendorf 5417C)

1. Resuspend column material by using a vortex
2. Loosen cap ¼ turn and snap off the bottom closure
3. Place the column in a 2 mL collection tube (not provided)
4. Pre-centrifuge the column for 1 minute at 20,800 x g (i.e. max speed of a typically tabletop microcentrifuge)
5. Discard flow-through and column cap, but re-use collection tube
6. Add 300µL ultrapure water to the column and centrifuge for 1 min. max speed using a tabletop centrifuge
7. Discard collection tube and flow-through
8. Place column into a new DNase free 1.5 mL micro-centrifuge tube (not provided)
9. Add ULS-labeled sample onto the center of the column bed
10. Centrifuge column for 1 min. at max speed using a tabletop centrifuge
11. Flow-through contains the purified labeled DNA
12. At this point the degree of labeling (DoL) can be measured by using the DoL calculator provided at www.kreatech.com

Note: we ***highly recommend*** that the DoL is measured after each labeling reaction. It is advised to use the labeled DNA for array CGH hybridizations only when the DoL value is between 1.0 – 2.5 % (optimal DoL value is 1.5-2.0%). Values outside this range might produce low microarray signals due to low fluorescence (when DoL <1.0%) or quenching (when DoL >2.5%).

V. Guidelines for Microarray Hybridization

ULS labeled and purified fluorescent genomic DNA can be used with different types of microarrays. The ULS arrayCGH labeling kit contains formamide based hybridization buffer. This buffer was optimized on BAC arrays printed on aminosilane coated slides. Slides with other surface chemistries may need special treatment in which case we recommend that you paying attention to the instructions provided with your slide of choice. It may be useful to test our hybridization buffer alongside the buffer supplied with your slide.

a. Preparation of Labeled Material for Hybridization

Labeled samples need to be concentrated before hybridization; this can be done via precipitation as described below or under a vacuum.

1. For dual color assays, pool the labeled samples
2. Add 12.5 x excess C_0t-1 DNA (e.g. 50 µg in one hybridization when using 2 µg reference and 2 µg sample)

Concentration by precipitation

1. Add ¼ volume of 10 M amonium acetate and 2.5 volumes of 100% ethanol
2. Incubate for 30 minutes at –20ºC
3. Spin at max speed (20,800 x g) at 4ºC for 30 minutes
4. Remove supernatant and dry pellet briefly. This pellet should have a purple color when both genomic and reference DNA are labeled optimal

Preparation of hybridization mixture

To dissolve the precipitated material, add the appropriate amount of water, SDS and tRNA solution depending on the desired hybridization volume (see table below) and incubate for 10 minutes at room temperature (ultrapure water in this step can be replaced by KREAblock, which can be beneficial in reducing background) Finalize the hybridization mixture according to the table below by adding the appropriate amount of a KREAHYB-CGH.

Pelleted target and C_0t-1 DNA					
ultrapure water (or KREA*block*™ see above)	4.6	6.9	9.2	13.8	µL
20% SDS	5.4	8.1	10.8	16.2	µL
Yeast tRNA (100 µg/µL)	4.0	6.0	8.0	12.0	µL
Incubate for 10 minutes at room temperature					
KREAHYB-CGH	26	39	52	78	µL
End volume	40	60	80	120	µL

b. Hybridization work flow

Required Reagents

PN buffer

- Take 473.5 mL 0.2 M Na_2HPO_4, set pH to 8.0 with 0.2 M NaH_2PO_4 (~ 26.5 mL), adjust volume to 1.0 L with H_2O

0.2 M Na_2HPO_4:

- Dissolve 35.6 g $Na_2HPO4{\cdot}2H_2O$ in 1.0 L H_2O,
- Add 1 mL NP-40 (Igepal CA-630)

0.2 M NaH_2PO_4:

- Dissolve 27.6 g $NaH_2PO_4{\cdot}1H_2O$ in 1.0 L H_2O,
- Add 1 mL NP-40 (Igepal CA-630)

50% formamide / 2 x SSC

- Take 500 mL formamide, add 100 mL 20 x SSC (pH 7.0) and add 400 mL H_2O

Pre Hybridization

1. Denature the hybridization mixture 15 minutes at 70℃ in a water bath
2. To block repetitive sequences with C_0t-1 DNA incubate the hybridization mixture at least 30 minutes at 37℃ i n a water bath
3. Pretreat the microarray according to manufacturer's instructions
4. Add the whole hybridization solution to the microarray and hybridize overnight at 37℃

Post Hybridization

Washing

1. Preheat the 50% formamide / 2 x SSC wash solution at 45℃ > 30 minutes
2. Wash the slides 15 minutes with 50% formamide / 2 x SSC wash solution at 45℃ in a shaking water bath
3. Wash with PN-buffer for 15 minutes at room temperature
4. Dip the slides briefly in water
5. Dry the slides immediately either by centrifugation for 5 minutes in 50 ml tubes or alternatively dry slides with compressed nitrogen
6. Store the slides dry and in the dark before scanning

For more information regarding this product or our ULS technology we invite you visit our website www.kreatech.com, where you will find a wealth of information that may be useful in your experiment such as protocols on sample deparaffinization (for FFPE samples), DNA Extraction, DNA Fragmentation etc.

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