LABELING KIT

ULS™

ULS™ arrayCGH Labeling Kit

For 10 dual labeling reactions
(20µg per color)

Product code
EA-001/EA-005/EA-007

For laboratory use only
Research purposes only

KREATECH
BIOTECHNOLOGY

Instruction manual

KREATECH's ULS™ arrayCGH Labeling Kit

This kit is intended for RESEARCH USE ONLY. IT IS NOT INTENDED FOR DIAGNOSTIC APPLICATIONS and/or COMMERCIAL PURPOSES.

Important

Open the kit immediately and store all components as instructed on page 7

- Read the entire Instruction manual before starting your experiment.
- Do not mix reagents from different kits.
- During the preparation of reagents and throughout the entire procedure please observe the safety regulations issued for laboratories concerning handling of samples.
- Dispose of reagents according to relevant local regulations. Take appropriate safety precautions such as wearing a lab coat, gloves and eye protection.

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A. Assay Materials

I. Components and Storage

Component	Amount	Storage
Cyanine3/Cy3/DY547 - ULS™	10 µL	4°C
Cyanine5/Cy5/DY647 - ULS™	10 µL	4°C
10 x Labeling Solution	100 µL	4°C
KREA*pure*™ columns	20 pcs	4°C
KREA*block*™	1 mL	–20°C*
KREAHYB-CGH	2 mL	4°C

** KREAblock is shipped at 4°C, store at –20°C upon delivery.*

II. Reagents not Included in Kit

Reagents are not supplied but may be required. All reagents should be of molecular biology grade and free of nucleases. Reference DNA, Fragmentation reagents, AmAc (10 M), Human C_0t-1 DNA (Invitrogen or Roche), Yeast tRNA (Invitrogen, # 15401-029).

B. General Information

I. Background

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

II. The ULS arrayCGH Labeling Kit

The ULS arrayCGH 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, cost-efficient and highly reproducible labeling for arrayCGH analysis.

The ULS arrayCGH Labeling Kit provides enough reagents for 10 dual hybridizations of 2 µg of genomic DNA per color.

The ULS arrayCGH Labeling Kit can also be used to label 20 µg per color of amplified genomic DNA which is generated using unmodified nucleotides. The amount of ULS required depends on the amount of amplified DNA required for hybridization. The scale of the labeling reaction can be adjusted however the ratio of DNA to ULS reagent needs to be precise and the same as is stated in the protocol.

III. Principle of ULS Labeling

The proprietary ULS technology is based on the stable binding properties of a platinum complex to biomolecules. The ULS molecule itself consists of a platinum complex, a detectable molecule and a leaving group which is displaced upon reaction with the target. This ULS molecule forms a co-ordinative bond, firmly coupling the ULS to the target. ULS labels DNA, and RNA by binding to the N7 position of guanine. In proteins, ULS binds to nitrogen and sulphur containing side chains of methionine, cysteine and histidine (see figure inside back cover).
ULS is available coupled to a variety of labels and haptens, including fluorochromes and biotin.
ULS thereby enables one-step non-enzymatic labeling of nucleic acids to be achieved within 30 minutes. ULS labeling can be performed with or without enzymatic amplification, prior too labeling.

IV. ULS arrayCGH Labeling Process

The procedure of genomic DNA labeling with the ULS arrayCGH Labeling Kit is as follows:

1. Genomic DNA is extracted from cells or tissues of interest (If required the genomic DNA can be amplified using natural unmodified nucleotides followed by ULS labeling)
2. Sample DNA is fragmented
3. Sample DNA, in parallel with reference DNA is non-enzymatically labeled with ULS reagent
4. The labeled DNA is purified using a KREA*pure* column
5. The labeled DNA is hybridized to a microarray in an overnight incubation
6. A laser detection system is required to scan and report the relative quantity of the two dyes at any given spot on the microarray
7. Finally, computer-imaging software processes the differential scanning data to analyze the complete array

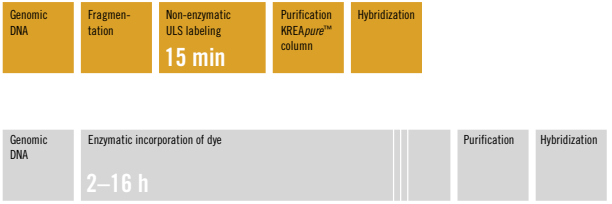

Figure 2. Labeling with ULS is achieved within minutes instead of hours

V. Schematic Overview of the ULS arrayCGH Labeling Process

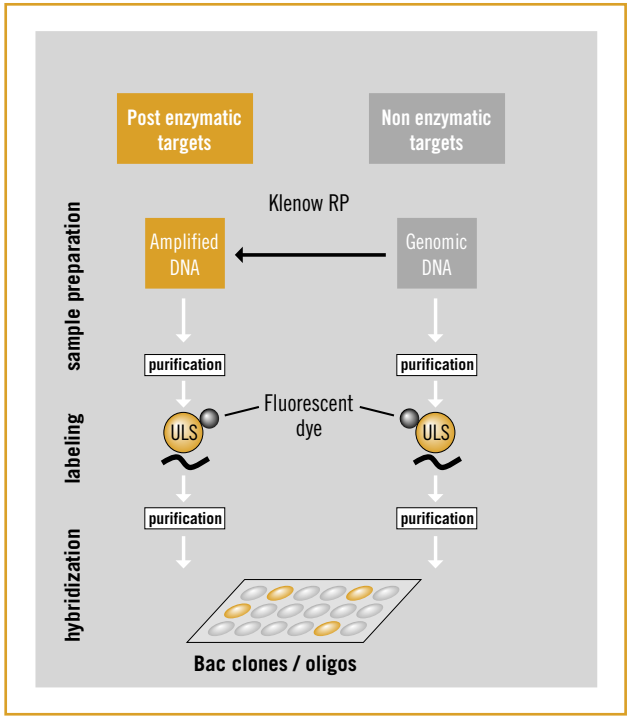

Figure 3. ULS labeling can be performed with or without enzymatic amplification prior to labeling.

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 >1.6 and <2.0.
Furthermore, DNA samples need to be clean of divalent cations (e.g. Mg^{2+}) salt and other (wash) buffer components which could disturb the labeling efficiency.
Note: Be aware that some components in silica based purification systems inhibit the ULS reaction. A final wash step using 80% ethanol (PA) before elution should prevent this. Alternatives: alcohol precipitation of eluted material or cleanup using an EDTA, sephadex combination.

II. DNA Amplification (optional)

No amplification is needed

If the amount of target for your experiment is sufficient proceed to fragmentation of DNA. (Note: it is recommended to have some DNA for gel analysis after fragmentation)

Amplification needed

If the amount of isolated DNA is insufficient the sample may be amplified using a commercial kit. When labeling with ULS, amplified material is generated using natural unmodified nucleotides.
Note:

- Purify using standard DNA purification systems (note C 1 above)
- Measure OD_{260} to determine DNA concentration

III. DNA Fragmentation

If the material is amplified using a commercial kit then fragment length may be ok, however it is advised to check if the resulting fragments need further fragmentation.

When using natural genomic DNA as sample, fragmentation is always necessary.

Fragmentation

(Note: we recommend that you have enough DNA for testing the fragment length on gel following fragmentation).

Fragment your DNA according to your own protocol. We recommend using sonication. With sonication all DNA is fragmented to similar size, and avoids the possibility of over fragmentation. Fragmentation by restriction enzyme (4-cutter) or DNAse is also possible. Be aware that after fragmentation with restriction enzymes and before ULS labeling it is necessary to purify the DNA so that it is free from divalent cations and other contaminants which may inhibit the ULS labeling reaction (See note C I).

Note: In order to obtain optimal labeling densities, it is very important to determine the DNA concentration before starting the labeling procedure. The final concentration of the DNA is important for the proceeding labeling reaction. In general suboptimal modification degrees of the labeled material are achieved if the final concentration of the DNA in the labeling mixture is below 50ng/µL.

IV. DNA Labeling

a. ULS Labeling Procedure

Ratio of ULS per microgram target: 1µL of Cyanine3/Cy5/DY547-ULS or Cyanine5/Cy5/DY647-ULS 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 Cyanine3/Cy3/DY547-ULS or Cyanine5/Cy5/DY647 ULS to exactly 2µg DNA
2. Adjust with DNase-free water to final volume (preferably end volume of the labeling mixture is 20µL; see example)
3. Label sample by incubation for 30 minutes at 85°C
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
Cyanine/Cy/DY - ULS	1.0 µL
10 x labeling solution	2.0 µL
Total volume	20.0 µL

b. Dye Removal Using KREA*pure* Columns

Removal of free ULS label using KREA*pure* columns
(20800 g is equivalent to 14,000 rpm on eppendorf 5417C)

1. Resuspend column material by vortexing
2. Loosen cap ¼ turn and snap off the bottom closure
3. Place the column in a 2mL collection tube
4. Pre-spin the column for 1 minute at 20800 g
5. Discard flow-through and re-use collection tube
6. Wash the columns with 300µL DNase free water
7. Discard flow-through and collection tube

8. Put column in a new (DNase free) micro centrifuge tube
9. Add ULS-labeled DNA on column
10. Spin column for 1 minute at 20800g
11. Flow through is purified material
12. At this point the degree of labeling (DOL) can be measured (see page 20)

Your DNA sample is now labeled, purified and ready for hybridization.

V. Guidelines for Microarray Hybridization

We provide the following guidelines to users of the ULS arrayCGH Labeling Kit: ULS labeled and purified fluorescent genomic DNA can be used with different types of microarrays. The ULS arrayCGH labeling kit comes supplied with its own formamide based hybridization buffer, this buffer was optimized on aminosilane coated slides. Slides with other surface chemistries may need special treatment in which case we recommend that you consider the instructions provided with your chosen slide. 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.

- Pool the labeled samples (e.g. sample Cy5-ULS and reference Cy3-ULS)
- 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 AmAc and 2.5 volumes of 100% ethanol
2. Incubate for 30 minutes at –20°C
3. Spin at max speed (14,000 rpm) at 4°C 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

5. 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 (water in this step can be replaced by KREA*block*, which can be beneficial in reducing background)
6. Finalize the hybridization mixture according to the table below by adding the appropriate amount of a KREAHYB-CGH (formamide based) depending on the desired hybridization volume

Pelleted target and C_0t-1 DNA					
H_2O (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

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_2HPO_4-$2H_2O$ 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_4-1H2O 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°C in a water bath
2. To block repetitive sequences with C0t1 DNA in your sample incubate the hybridization mixture at least 30 minutes at 37°C in a water bath
3. Pretreat your DNA microarray according to manufacturer's instructions
4. Add the whole hybridization solution to the microarray and hybridize overnight at 37°C

Post Hybridization

Washing

1. Preheat the 50% formamide/ 2 x SSC wash solution at 45°C > 30 minutes
2. Wash the slides 15 minutes with 50% formamide / 2 x SSC wash solution at 45°C 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

D. Trouble Shooting

I. DNA Preparation and Analysis

Problem	Possible cause and remedy
OD_{260} not within parameters	Cause: Impure DNA. Remedy: Clean DNA by commercial clean-up kit or precipitate DNA and dissolve again.
Not enough material	Remedy: Amplify DNA with a commercial amplification kit.

II. ULS Labeling

Problem	Possible cause and remedy
Degree of labeling too low	Cause: There maybe salt present which disturb labeling. Remedy: Clean up DNA and ensure final 80% ethanol wash step is used with silica based columns. Cause: Wrong input DNA. Remedy: Re-measure DNA and add exact amount to reaction mixture.
Degree of labeling too high	Cause: Wrong input DNA. Remedy: Re-measure DNA and add exact amount to reaction mixture.
Pellet not purple, but red or blue	Cause: One of the labeling procedures was not efficient. Remedy: See above.
Pellet white	Cause: None of the labeling procedures were efficient. Remedy: See above.

III. Array Hybridization and Detection

Problem	Possible Cause And Remedy
Background on the slide	Remedy: Add KREA*block* in the hybridization mixture.
No signal at all	Cause: Too little sample hybridized. Remedy: Monitor amount of sample to be hybridized. Cause: Labeling procedure not efficient. Remedy: See above under ULS labeling. Cause: Precipitation was not efficient. Remedy: Check for purple pellet.
Aspecific signal	Cause: Suboptimal hybridization conditions. Remedy: Ensure C_0t-1 DNA added and pre-treatment of the slides is performed.

E. Appendix

I. Determination of DNA Quality

- Measure A_{260} and A_{280} nm using a spectrophotometer and calculate $OD_{260/280}$. For good quality DNA this value should be between 1.6 and 2.0

II. Determination of the Degree of Labeling (DOL)

- Measure A_{260} and A_{550} for determining the DOL of Cyanine3/Cy3/DY547-ULS labeled DNA
- Measure A_{260} and A_{650} for determining the DOL of Cyanine5/Cy5/DY647-ULS labeled DNA

$$\text{ng / µL} = \frac{A_{260} \text{ *dilution factor* } 50}{\text{cuvet length (in cm)}}$$

$$\text{pmol / µL} = \frac{A_{\text{dye at max}} \text{* dilution factor}}{\text{cuvet length } * \varepsilon_{\text{dye}} * 10^{-6}}$$

εdye Cyanine3/Cy3/DY547 Reagent = 150.000

εdye Cyanine5/Cy5/DY647 Reagent = 250.000

Degree of labeling (amount of dyes per 100 nucleotides)

$$\text{Labeling \%} = \frac{340 * \text{pmol}_{\text{dye}}}{\text{ng}_{\text{ nucleic acid}} * 1000 \text{ x } 100\%}$$

- Differences in genomic DNA purity can cause slight variations in the DOL. As a guide an optimal DOL for arrayCGH lies between 1.0 % and 2.5 %

F. References

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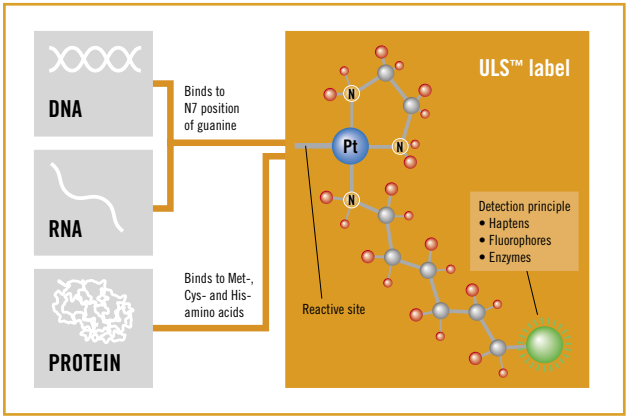
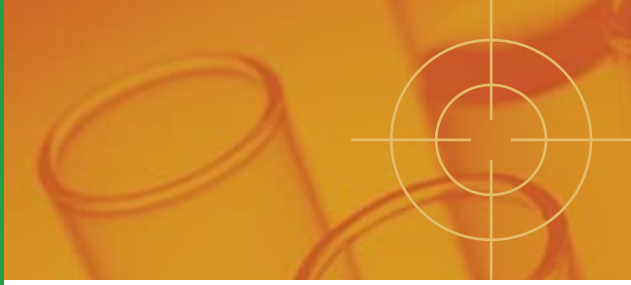


Figure. ULS™ labels RNA by forming a coordinative bond on the N7 position of guanine.



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