LABELING KIT

ULS™

ULS™ aRNA Labeling Kit
- **with Biotin-ULS**
- **with Biotin-ULS and Streptavidin Phycoerythrin**
- **with Biotin-ULS - for Affymetrix® Genechips®**
- **with Biotin-ULS and Streptavidin Phycoerythrin - for Affymetrix® Genechips®**

For 25 labeling reactions

Product code
EA-010/EA-017/EA-018/EA-019

For laboratory use only
Research purposes only

KREATECH BIOTECHNOLOGY

Instruction manual

KREATECH's ULS™ aRNA Labeling Kits

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 6

- 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

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

Component	Concentration	Quantity	Storage Conditions
BIO-ULS	-	25 reactions	4°C
10 x Labeling Buffer	-	100 µL	4°C
KREA*pure* columns	-	25 pcs	4°C
KREA*block*	-	1.5 mL	-20°C*
In EA-017/EA-019 Streptavidin Phycoerythrin	1mg/mL	300 µL	4°C

II. Reagents and Buffers not Included in Kit

Fragmentation reagents (Ambion Cat # 8740)

B. General Information

I. Background

Gene expression profiling using DNA-microarrays has been growing rapidly over the last decade. When working with material from fine needle biopsy, laser capture micro-dissection material and with patient material in general, the amount of target material is limited. This has made it necessary to carry out target amplification, generally by linear amplification based on a protocol first described by van Gelder and Eberwine[1]. This procedure is based on the reverse transcription from mRNA into double stranded cDNA using an Oligo(dT) primer containing a T7 RNA polymerase promoter sequence. Linear amplification is achieved during the subsequent IVT reaction using T7 polymerase with cDNA as a functional template.

The ULS aRNA Labeling Kits have been designed to enable the generation of unmodified amplified aRNA using natural nucleotides, which can then be labeled with BIO-ULS. There are four different formats available:

Product Code	Name	Labeling Reactions	aRNA per ULS Labeling Reaction
	aRNA Labeling Kits		
EA-018	with Biotin-ULS	25	≤ 10µg
EA-019	with Biotin-ULS and SA-PE	25	≤ 10µg
EA-010	with Biotin-ULS for Affymetrix® Genechips®	25	≤ 20µg
EA-017	with Biotin-ULS and SA-PE for Affymetrix® Genechips®	25	≤ 20µg

In addition to ULS Labeling Kits, Kreatech Biotechnology also provides kits to amplify your RNA before labeling: the RNA ampULSe: Amplification and Labeling Kits. These kits employ Ambion's widely used MessageAmp™II aRNA amplification reagents followed by biotin labeling with KREATECH's proprietary ULS technology. There are four available formats:

Product Code	Name	Labeling Reactions	aRNA per ULS Labeling Reaction
	Amplification and Labeling Kits		
GEA-006	RNA ampULSe – amplification and labeling Kit with Biotin-ULS	20	≤ 10µg
GEA-007	RNA ampULSe – amplification and labeling Kit with Biotin-ULS and SAPE	20	≤ 10µg
GEA-004	RNA ampULSe – amplification and labeling Kit with Biotin-ULS for Affymetrix® Genechips®	20	≤ 20µg
GEA-005	RNA ampULSe – amplification and labeling Kit with Biotin-ULS and SA-PE for Affymetrix® Genechips®	20	≤ 20µg

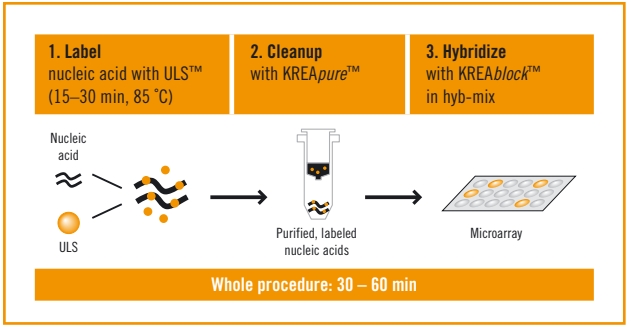
II. Principle of ULS Labeling

The proprietary ULS technology is based on the stable binding properties of platinum to biomolecules. The ULS molecule 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.

III. ULS aRNA Labeling Process in Gene Expression Analysis

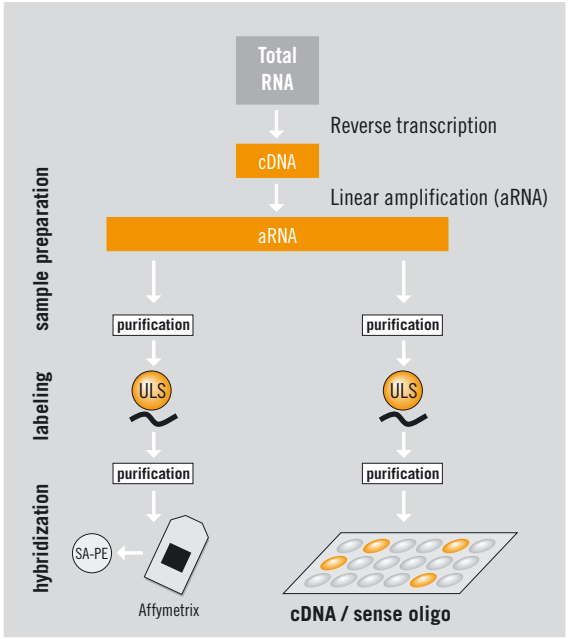
1. Generation of aRNA from isolated total RNA via linear amplification using natural unmodified nucleotides
2. Non-enymatic labeling of aRNA with ULS reagent (30 min)
3. Purification of the labeled aRNA with the KREA*pure* column
4. Fragmentation of the labeled aRNA
5. Hybridization of the fragmented and labeled aRNA to a microarray in the presence of KREA*block*

Figure 2: A 30-60 minute protocol for DNA microarray applications



IV. Schematic Overview of the ULS aRNA Biotin Labeling Process

Figure 3. Schematic Overview of ULS aRNA Biotin Labeling Process.



C. Protocol

I. Total RNA Isolation

A wide variety of RNA isolation techniques are available, e.g. using Trizol (Invitrogen) extraction followed by RNeasy column purification (QIAGEN). Irrespective of which isolation procedure is used the RNA material should be free from DNA and other contaminants. Assessment of the purity and yield of your RNA should be carried out by

- Running your RNA on a 1% agarose gel. The integrity of the total RNA is determined by observing the ribosomal bands
- Determining the $OD_{260/280/230}$.

For all RNAs $OD_{260/280}$ should be >1.9 and $OD_{260/230}$ should be >2.1

II. Linear Amplification

ULS technology allows labeling of aRNA generated from a variety of commercially available kits, e.g. MessageAmp™ aRNA amplification kit (Ambion), MEGAscript™ T7 kit (Ambion).

Important! With the ULS protocol, amplification is carried out prior to labeling with the detection moieties. Enzymatic reactions should be carried out using only unmodified nucleotides. This results in better yields, longer fragments and a more stable amplified RNA sample.

Furthermore, aRNA samples need to be clean of divalent cations (e.g. Mg^{2+}), salts and other (wash) buffer components which could disturb the labeling efficiency.
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.

III. aRNA Labeling

a. ULS-Labeling Procedure

Briefly spin all required reagents to collect contents of tubes

1. Take the required amount of your unmodified aRNA ensure final concentration in labeling reaction is above 50 ng/µL. Optimal modification degrees of the labeled material are not achieved if final concentration of the aRNA in the labeling mixtures is below 50 ng/µL)
2. Add 0.25 µL of BIO-ULS per 1µg of aRNA
3. Add 1/10 volume of 10x labeling solution
4. Adjust with RNase-free water to final volume and mix by pipetting (see example set-up below)
5. Label sample by incubation for 30 minutes at 85°C
6. Place samples on ice, spin down to collect contents of tube before proceeding with purification using the KREA*pure* columns

Example of BIO-ULS labeling of aRNA.

The important factors to consider when setting up the labeling experiments are:

1. To ensure the concentration of nucleic acid in the labeling reaction is > 50ng/µL. i.e. if labeling 4µg in 30µL as in the example set up below the nucleic acid concentration = 133ng/µL.
2. The KREA*pure* column capacity is 50 µL. If the labeling reaction volume exceeds this then concentration will be necessary before purification over the KREA*pure* column.

	4µg	8µg	20µg
aRNA + RNAse free water	26µL	25µL	22µL
BIO-ULS	1µL	2µL	5µL
10 x labeling solution	3µL	3µL	3µL
Total Volume	30µL	30µL	30µL

b. Dye Removal Using KREA*pure* Columns

Removal of free ULS label using KREA*pure* columns

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

1. Resuspend column material by vortexing
2. Loosen cap 1/4 turn and snap off the bottom closure
3. Place the column in a 2 mL collection tube
4. Pre-spin the column for 1 minute at 20800 x g
5. Discard flow-through and re-use collection tube
6. Wash the column with 300 µL RNase free water
7. Discard collection tube and flow-through
8. Put column in a new (RNase free) 1.5 mL micro centrifuge tube
9. Add ULS-labeled aRNA on to column bed
10. Spin column for 1 minute at 20800 x g
11. Flow through is purified labeled aRNA

c. aRNA Fragmentation (reagents available from Ambion (cat#8740))

1. Transfer labeled sample to a microfuge tube and add 1/10 volume of 10 x fragmentation buffer (Ambion) to decrease the fragment size to 60-200 bases. (e.g. 4 µL in final volume of 40 µL)
2. Incubate at 70°C for 15 minutes
3. Spin the vial briefly and add 1µL of stop solution (Ambion), mix by pipetting (the labeled aRNA can form aggregates which dissolve by pipetting) and place on ice until further use

IV. Preparation of Labeled Material for Hybridization

1. KREA*block* should be added to 1/4 final volume of the hybridization mixture (e.g. 25 µL of KREA*block* in a 100 µL hybridization volume)
2. Hybridize and wash slides according to own protocol

Products EA-017 and EA-019 provide SA-PE (Streptavidin phycoerythrin) for detection.The kits provide enough SA-PE for at least 25 Microarrays or Genechips®

V. Detection using Streptavidin phycoerythrin

a. To use the SA-PE in an Affymetrix® Genechip® setting we suggest referring to the Affymetrix protocol
b. For use in other DNA microarray platforms we recommend a final concentration of 10 µL/mL of SA-PE in your final SA-PE stain solution

D. Trouble Shooting

I. Total RNA and aRNA Preparation and Analysis

Problem	Possible Reasons and Suggestions
OD_{260} not within parameters	Cause: Impure RNA Remedy: Repeat RNA clean-up using a commercial kit or precipitate RNA and dissolve again

II. Labeling, Array Hybridization and Detection

Problem	Possible Reasons and Suggestions
Low Signals	Inefficient labeling of aRNA Cause: There may be salt present which disturbs labeling Remedy: Clean up aRNA and ensure final 80% ethanol wash step is used with silica based columns (see C II) Cause: Incorrect ratio of labeling reagent to aRNA Remedy: Ensure use of instructed amount of ULS per µg of aRNA Remedy: Ensure concentration of the labeling reaction is above 50 ng/µL

Problem	Possible Reasons and Suggestions
Background on the slide	Cause: Too much sample added to microarray Remedy: Reduce sample amount Cause: Insufficient blocking Remedy: Add more or alternative blockers to microarray Cause: Too high concentration of detection antibody Remedy: Dilute detection antibody

E. References

1. Van Gelder RN et al. (1990), Proc Natl Acad Sci USA 87: 1663–1667

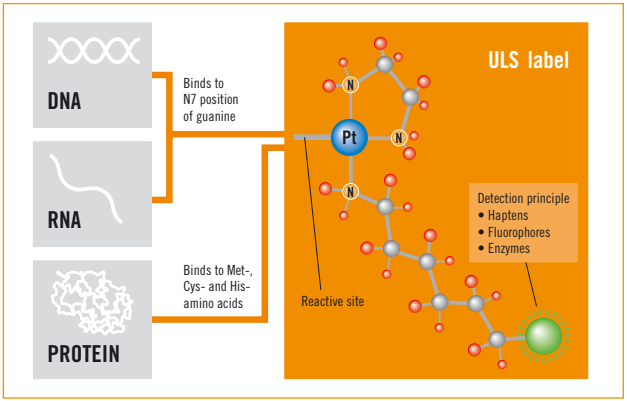


Figure. ULS labels DNA, RNA and proteins.

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