LABELING KIT

ULS™

Platinum*Bright*™: Nucleic Acid Labeling Kit

For 20 labeling reactions

(Labels 20µg)

Product code

GLK-001/GLK-002/GLK-003/GLK-004/GLK-005/
GLK-006/GLK-007

For laboratory use only

Research purposes only

Instruction manual

KREATECH's Platinum*Bright*™: Nucleic Acid 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 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.
- MSDS on request.

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A. Assay Materials

I. Components and Storage

The contents of this Platinum*Bright* kit are sufficient for labeling max. 20 µg input nucleic acid template according to the standard labeling protocol.

Amount	Component	Storage
40µL	ULS hapten/dye	4°C
100uL	10 x labeling solution	4°C
20 pcs	KREA*pure*™	4°C

II. Quality Control

In the standard QC procedure 1 µg of non-labeled, fragmented, Repeat DNA, Unique DNA and Whole Chromosome Paint DNA are labeled with ULS following the standard protocol. FISH hybridization is carried out followed by direct detection, except with the biotin-ULS labeled probes where detection is in-direct with strep-avidin-Cy3.

B. General Information

I. Product Description and Background

The Platinum*Bright* family of labeling products is based on a platinum complex that binds to biomolecules, called the Universal Linkage System (ULS).
The Pt-compound has two free binding sites, one of which is used to bind a marker group, thus forming a marker/platinum complex. The other binding site is used to link the complex to the N7 position of the guanine base of nucleic acids.

Intended Use

KREATECH's Platinum*Bright*: nucleic acid labeling kits are intended for the non-enzymatic labeling of single-stranded, double-stranded, linear or supercoiled DNA, oligonucleotides, RNA or PCR products.

The Platinum*Bright* kits offer the opportunity to label 20 µg target nucleic acid material. In addition, the kit contains a simple labeling protocol and sufficient reagents for labeling the nucleic acid template of your choice. The nucleic acid can be DNA or RNA in any form either from natural, enzymatic or synthetic origin.

Fluorescent dyes/haptens available within the PlatinumBright nucleic acid labeling kits

Catalog #	Product name	ULS dyes/ hapten	Abs (nm)	Em (nm)	Ext coefficient
GLK-001	Platinum*Bright* 495 Green	Fluorescein-ULS	495	517	83,000
GLK-002	Platinum*Bright* 547 Red/Orange	Dyomics547-ULS	547	565	150,000
GLK-003	Platinum*Bright* 647 Infrared	Dyomics647-ULS	647	665	250,000
GLK-004	Platinum*Bright* 550 Red/Orange	Rhodamine-ULS	550	573	91,000
GLK-005	Platinum*Bright* 570 Red/Orange	d-Red-ULS	570	591	129,000
GLK-006	Platinum*Bright* 415 Blue	Dyomics415-ULS	415	472	56,000
GLK-007	Platinum*Bright*	Biotin-ULS	--	--	--

II. Principle of the Universal Linkage System (ULS)

The proprietary ULS technology is based on the stable binding properties of a platinum complex 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. Critical Parameters for a Successful Labeling

- All nucleic acid samples need to be clean of divalent cations (e.g. Mg^{2+}) salt and other (wash) buffer components which could disturb the labeling efficiency
  - Be aware that some components in silica based purification systems may inhibit the ULS reaction. A final wash step using 80% ethanol (PA) before elution prevents this. Alternatives: alcohol precipitation of eluted material or cleanup using an EDTA, sephadex combination
- Assessment of the purity and yield of your DNA or RNA should be carried out by
- Running your RNA on a 1% agarose gel
- Determining the OD_{260}
- Labeling efficiency is optimal at concentrations above 50ng/uL. (Labeling will occur at lower concentration but with lower efficiency (try and compensate by adding more ULS)

- The labeling volume is flexible as long as the DNA concentration is not lower as 50 ng/µL and the amount of ULS added is adjusted to the amount of input DNA
- High Tris-HCl concentrations (>40 mM) or EDTA (>5 mM), Mg-acetate (>1 mM), NaCl (> 100 mM) and restriction enzyme digestion buffers have a rate-limiting effect on the labeling reaction and should therefore be removed prior to labeling
- For in situ hybridization, the size of input DNA is recommended to be between 100 and 300 base pairs (of chromosome specific libraries: 200 - 2000 base pairs). This can be achieved by sonication, DNase treatment (DNA) or controlled alkaline hydrolysis (RNA), prior to labeling

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C. Applications of the Platinum*Bright*; Nucleic Acid Labeling Kits

(Specific advice available upon request)

Applications:

- In situ hybridization
  - ULS labeling of DNA for in situ hybridization (DISH)
  - ULS labeling of RNA for in situ hybridization (RISH)
  - ULS labeling of DNA for Fluorescent in situ hybridization (FISH)
  - Multicolor fluorescence in situ hybridization (MFISH)
  - ULS labeling of DNA for Comparative Genomic Hybridization (CGH)
- Filter hybridization
  - Spot blot analysis
  - Dot blot hybridization
  - Southern blot analysis
  - Northern blot analysis

- Tissue arrays
  - ULS labeling of DNA for in situ hybridization (DISH)
  - ULS labeling of RNA for in situ hybridization (RISH)
  - ULS labeling of DNA for Fluorescent in situ hybridization (FISH)
- ChIP on Chip
  - Labeling of promoter fragments after Chromatin immuno precipitation analyzed on DNA arrays (ChIP on Chip experiments)

Nucleic acid targets:

- DNA:
  - Plasmids, cosmids, BACs, PACs, YACs .
  - PCR products (eg DOP-PCR products) and other enzymatically generated DNAs
  - Highly repetitive DNA sequences (e.g. satellite, centromeric and telomeric probes)
  - Synthetic DNA (oligonucleotides)
- RNA:
  - Total RNA
  - Messenger RNA
  - other RNA species
  - In Vitro transcribed RNA and other enzymatically generated RNAs

D. Protocols

I. Suggested Fragmentation Protocols

- If DNA fragmentation by DNase I will be carried out then this must be done before ULS labeling. If fragmenting DNA by sonication then this may be carried out before or after ULS labeling

For in situ hybridization the optimal probe fragment size is between 100-300 bp. Probes over 1 kb will generate a spotted background in in situ hybridization. Because most PCR generated probes are smaller than 1 kb, these probes can be labeled without fragmentation. Other probes such as plasmids, cosmids, YACs, PACs and BACs must be fragmented prior to labeling with ULS. Fragmentation can be performed by sonication or DNase I treatment.

a. Suggested Fragmentation by Sonication

1. Prepare at least 100µL of DNA solution in 10 mM Tris-HCl, pH 8.0, 0.3 mM EDTA (TE) with a minimal DNA concentration of 20ng/µL
2. Sonicate for 3 cycles of 1 min. each at amplitude 5, keep sample on ice. Cool the DNA solution on ice for 1 min. after each cycle and spin down the DNA solution in a microcentrifuge for 5 sec. at maximum speed before the second/third sonication step
3. Check the fragment length by electrophoresis on a 1% agarose gel
4. Label the DNA according to the labeling protocol

b. Suggested Fragmentation by DNase I Treatment

1. Prepare DNase I stock solution: dissolve 1mg of DNase I (Roche # 104 159, approx. 2000 units/mg) in 1mL of 5 mM NaAc pH 5.2, 1mM CaCl2, 50% glycerol. Make sure buffer is ice cold before adding the lyophilized DNase I. Mix by inversion. Do not vortex. Store stock DNase I solution at -20°C; avoid freeze-thaw cycles
2. Prepare 10´ nicking buffer: 50 mM Tris-HCl pH 7.5, 10 mM CaCl2, 10 mM MgCl2
3. Dilute the DNase I stock 1:5000 in 1´ nicking buffer. The buffer solution should be cold before the addition of the enzyme. These dilutions should be prepared immediately before use
4. Setup nicking reaction on ice as follows:
   1µg template DNA
   2.5µL 10´ nicking buffer
   3-5µL diluted DNase I
   H2O to 25µL
5. Incubate at 37°C for 10 minutes. Stop reaction by placing on ice and precipitate with ¼ volume of 10M NH4Ac and 2.5 volumes of 100% EtOH. Resuspend the Nuclease-free Water
6. Check the fragment length by electrophoresis on a 1% agarose gel

II. ULS Labeling

For labeling 1µg nucleic acid sample

Briefly spin all required reagents to collect contents of tubes

1. Take 1 µg of nucleic acids sample ensure final concentration in labeling reaction is above 50 ng/µL. (In general suboptimal modification degrees of the labeled material are achieved if final concentration of the nucleic acid sample in the labeling mixture is below 50 ng/µL)

2. Add 2µL of ULS label or per 1µg nucleic acid sample
3. Add 1/10 volume of 10x Labeling solution
4. Adjust with Nuclease-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 ULS labeling of 1 µg nucleic acid sample in 20µL

Nucleic acid sample in Nuclease-free Water	16 µL
ULS dye	2 µL
10 x labeling solution	2 µL
Total volume	20 µL

Dye removal 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 2 mL 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 column with 300 µL RNase free water
7. Discard collection tube and flow-through
8. Put column in a new (nuclease free) 1.5 mL micro centrifuge tube
9. Add ULS-labeled nucleic acid on to column bed
10. Spin column for 1 minute at 20800 g
11. Flow through is purified labeled nucleic acid sample
    At this point the degree of labeling (DOL) can be measured (see appendix)

E. Trouble Shooting

Problem	Possible Cause	Suggestions
Labeling of template too low	Amount of ULS added is not correct	Check the ratio of ULS/nucleic acid
	Concentration of template is lower than measured	Use at least a concentration of 50ng/μL of you nucleic acid sample
	Reaction temperature is too low	Make sure temperature is 85°C
	Template preparation is insufficiently pure	Purify DNA/RNA by phenol extraction and/or ethanol precipitation
	Sample contains too much NaCl, TRIS, EDTA or proteins	Purify DNA/RNA by phenol extraction, ethanol precipitation or a commercial nucleic acd purification column Note: with silica based columns it is important to add an 80% ethanol wash step to insure no buffer contaminants are in your sample

No hybridization signal	Hybridization and wash conditions are too stringent	Reduce temperature of hybridization Lower stringency of the wash buffer
	Labeled fragments are too large	Reduce size of probe by fragmentation prior to labeling to ensure proper hybridization of the probe (see fragmentation protocols)
	Labeling density is too low	Check your labeling density and if necessary add more ULS per µg nucleic acid
Unspecific hybridization signal (background)	Hybridization temperature is too low	Increase hybridization temperature and/or increase temperature during washing step
	Probe concentration is too high	Reduce probe concentration

F. Appendix

I. Degree of Labeling (DOL)

This kit is designed to give an optimal labeling density as determined in FISH applications. The ULS/nucleic acid ratio can be easily adjusted to optimize the labeling density for other applications if necessary.

II. Determination of the Degree of Labeling (DOL)

Measure A_{260} and Dye-Abs for determining the DOL of ULS labeled RNA/DNA.

$$ng / \mu L = \frac{A_{260} * \text{dilution factor} * 33\,\text{OLIGO}/40\,\text{RNA}/50\,\text{dsDNA}}{\text{cuvet length (in cm)}}$$

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

εdye: see page 7

Degree of labeling (amount of dyes per 100 nucleotides)

$$\text{Labeling \%} = \frac{340 * pmol_{dye}}{ng_{\text{nucleic acid}} * 1000} \times 100\%$$

G. References

1. Jelsma T. et al., Increased labeling of DNA probes for in situ hybridization with the Universal Linkage System (ULS®), *Journal of NIH Research*, Vol. 5, p. 82, 1994
2. Kottler M.L. et al., Subregional mapping of the human Gonadotropin-Releasing Hormone Receptor (GnRH-R) Gene to 4q between the markers D4S392 and D4S409, *Human Genetics*, Vol. 96, No. 4, p. 477-480, 1995
3. Van Belkum A. et al., Application of a new universal DNA labeling system in the PCR mediated diagnosis of Chlamydia trachomatis and human papillomavirus type 16 infection in cervical lesions, *Journal of Virological Methods*, Vol. 45, p. 189-200, 1993
4. Van Belkum A. et al., Non-isotopic labeling of DNA by newly developed hapten-containing platinum compounds, *BioTechniques*, Vol. 16, No 1, p. 148-153, 1994
5. Van Es R.M. et al., High sensitivity of in situ hybridization using sheep Fab fragments conjugates. *Journal of NIH Research*, Vol. 6, p. 88, 1994
6. Van Eijsden R. et al., Development of a rapid and highly sensitive labeling method of DNA and RNA probes for (fluorescent) in situ hybridization, *Abstract Book, San Diego AACC Conference*, 1996
7. Craig J. et al., Removal of repetitive sequences from FISH probes using PCR-assisted affinity chromatography, *Human Genetics* Vol. 100, p. 472-476, 1997
8. Tanke H. et al., New strategy for multicolor fluorescence in situ hybridization: COBRA: Combined Binary Ratio labeling. *European Journal of Human Genetics*. Accepted for publication

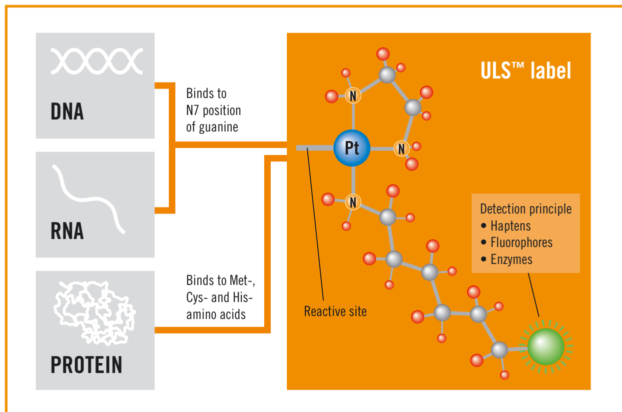

Figure. ULS™ labels RNA by forming a coordinative bond on the N7 position of guanine.

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