PlatinumBright™: 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
KREATECH’s PlatinumBright™: 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.

Limited Product Warranty – Patent Disclaimer

This warranty limits our liability to replacement of this product. No other warranties of any kind express or implied, including without limitation, implied warranties or fitness for a particular purpose, are provided by KREATECH. KCREATECH shall have no liability for any direct, indirect, consequential, or incidental damages arising out of use, or the inability to use this product.
This product or the use of this product is covered by US patents 5,580,990; 5,714,327; 5,985,566; 6,133,038; 6,338,943 and several foreign patents owned by KREATECH Biotechnology B.V, combined with USA, patent DE4445065 and related patents owned by Dyomics GmbH, Germany, or US patent number 5, 268, 486; 4,981,977 and other patents pending. KCREATECH is a trade name of KCREATECH Biotechnology B.V., ULSTM, KREApure™, and KREATblock™ are trademarks of KCREATECH Biotechnology B.V. KCREATECH’s Universal Linkage System are subject to patent protection. DY™ is a registered trademark of Dyomics GmbH, Jena, Germany.

Certain technologies and/or their methods of preparation, analysis or use may be covered by patents or other intellectual property rights held by others in certain countries. KCREATECH does not encourage or support the unauthorized or unlicensed use of any technology. Use of KCREATECH’s products is recommended only for technology for which the end-user has a license under proprietary rights of third parties or for technology for which a license is not required.

---

**Ordering Information and Technical Services**

**KCREATECH Biotechnology**

**Visiting address**
Vlierweg 20
1032 LG Amsterdam
The Netherlands

Phone: +31 20 691 9181
Fax: +31 20 696 3531
E-mail: info@kreatech.com
www.kreatech.com

**Postal address**
P.O. Box 37078
1030 AB Amsterdam
The Netherlands

**Technical Services**
techservices@kreatech.com
**Table of Contents**

Limited Product Warranty and Patent Disclaimer 3
Ordering Information’s and Technical Services 4

A. **Assay Materials** 6
   I. Components and Storage 6
   II. Quality Control 6

B. **General Information** 6
   I. Product Description and Background 6
   II. Principle of the Universal Linkage System (ULS) 8
   III. Critical Parameters for Successful Labeling 8

C. **Applications of the PlatinumBright: Nucleic Acid Labeling Kit** 9

D. **Protocols** 11
   I. Suggested Fragmentation Protocols 11
   II. ULS Labeling 12

E. **Trouble Shooting** 14

F. **Appendix** 16
   I. Degree of Labeling (DOL) 16
   II. Determination of the Degree of Labeling (DOL) 16

G. **References** 17
A. Assay Materials

I. Components and Storage
The contents of this PlatinumBright kit are sufficient for labeling max. 20 µg input nucleic acid template according to the standard labeling protocol.

<table>
<thead>
<tr>
<th>Amount</th>
<th>Component</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>40µL</td>
<td>ULS hapten/dye</td>
<td>4°C</td>
</tr>
<tr>
<td>100µL</td>
<td>10 x labeling solution</td>
<td>4°C</td>
</tr>
<tr>
<td>20 pcs</td>
<td>KREApure™</td>
<td>4°C</td>
</tr>
</tbody>
</table>

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 PlatinumBright 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 PlatinumBright: 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 PlatinumBright 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

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Product name</th>
<th>ULS dyes/hapten</th>
<th>Abs (nm)</th>
<th>Em (nm)</th>
<th>Ext coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLK-001</td>
<td>PlatinumBright 495 Green</td>
<td>Fluorescein-ULS</td>
<td>495</td>
<td>517</td>
<td>83,000</td>
</tr>
<tr>
<td>GLK-002</td>
<td>PlatinumBright 547 Red/orange</td>
<td>Dyomics547-ULS</td>
<td>547</td>
<td>565</td>
<td>150,000</td>
</tr>
<tr>
<td>GLK-003</td>
<td>PlatinumBright 647 Infrared</td>
<td>Dyomics647-ULS</td>
<td>647</td>
<td>665</td>
<td>250,000</td>
</tr>
<tr>
<td>GLK-004</td>
<td>PlatinumBright Infrared</td>
<td>Rhodamine-ULS</td>
<td>550</td>
<td>573</td>
<td>91,000</td>
</tr>
<tr>
<td>GLK-005</td>
<td>PlatinumBright 570 Red/orange</td>
<td>d-Red-ULS</td>
<td>570</td>
<td>591</td>
<td>129,000</td>
</tr>
<tr>
<td>GLK-006</td>
<td>PlatinumBright 415 Blue</td>
<td>Dyomics415-ULS</td>
<td>415</td>
<td>472</td>
<td>56,000</td>
</tr>
<tr>
<td>GLK-007</td>
<td>PlatinumBright</td>
<td>Biotin-ULS</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
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

C. Applications of the PlatinumBright; 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 KREApure 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 KREApure 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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeling of template too low</td>
<td>Amount of ULS added is not correct</td>
<td>Check the ratio of ULS/nucleic acid</td>
</tr>
<tr>
<td></td>
<td>Concentration of template is lower than measured</td>
<td>Use at least a concentration of 50ng/µL of you nucleic acid sample</td>
</tr>
<tr>
<td></td>
<td>Reaction temperature is too low</td>
<td>Make sure temperature is 85°C</td>
</tr>
<tr>
<td></td>
<td>Template preparation is insufficiently pure</td>
<td>Purify DNA/RNA by phenol extraction and/or ethanol precipitation</td>
</tr>
<tr>
<td></td>
<td>Sample contains too much NaCl, TRIS, EDTA or proteins</td>
<td>Purify DNA/RNA by phenol extraction, ethanol precipitation or a commercial nucleic acid purification column</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Note: with silica based columns it is important to add an 80% ethanol wash step to insure no buffer contaminants are in your sample</td>
</tr>
<tr>
<td>No hybridization signal</td>
<td>Hybridization and wash conditions are too stringent</td>
<td>Reduce temperature of hybridization and wash buffer</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Labeled fragments are too large</td>
<td>Reduce size of probe by fragmentation prior to labeling to ensure proper hybridization of the probe (see fragmentation protocols)</td>
<td></td>
</tr>
<tr>
<td>Labeling density is too low</td>
<td>Check your labeling density and if necessary add more ULS per µg nucleic acid</td>
<td></td>
</tr>
<tr>
<td>Unspecific hybridization signal (background)</td>
<td>Hybridization temperature is too low</td>
<td>Increase hybridization temperature and/or increase temperature during washing step</td>
</tr>
<tr>
<td>Probe concentration is too high</td>
<td>Reduce probe concentration</td>
<td></td>
</tr>
</tbody>
</table>
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.

\[
\text{ng / } \mu\text{L} = \frac{A_{260} \times \text{dilution factor} \times 33 \text{ Oligo/40RNA/50dsDNA}}{\text{cuvet length (in cm)}}
\]

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

$\varepsilon_{\text{dye}}$: see page 7

Degree of labeling (amount of dyes per 100 nucleotides)

\[
\text{Labeling } \% = \frac{340 \times \text{pmol}_{\text{dye}}}{\text{ng nucleic acid} \times 1000 \times 100}\%
\]
G. References


Figure. ULS™ labels RNA by forming a coordinative bond on the N7 position of guanine.
KREATECH Biotechnology
Vlierweg 20
1032 LG, Amsterdam
The Netherlands
Tel. +31 20 691 9181
Fax +31 20 696 3531
E-mail info@kreatech.com
www.kreatech.com