ULYSIS® Nucleic Acid Labeling Kits

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Ex/Em: See Table 1

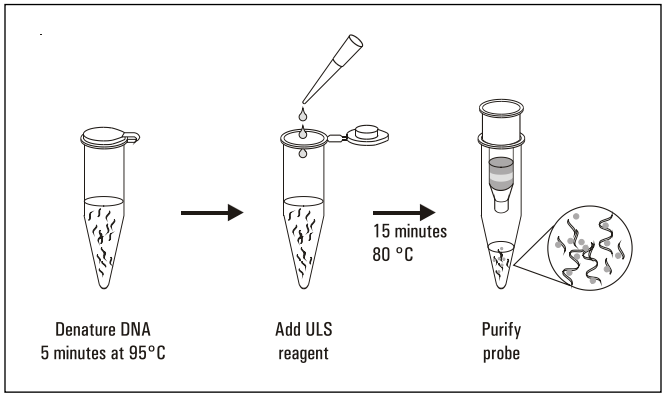

Figure 2. ULYSIS® nucleic acid labeling method.

Introduction

Molecular Probes is pleased to provide a non-enzymatic method for chemically labeling nucleic acids with our proprietary fluorescent dyes. In collaboration with KREATECH Diagnostics, we have developed a series of chemical labeling reagents that allow the end user to rapidly and easily couple our fluorescent dyes to purine bases in nucleic acid polymers. The method, the Universal Linkage System (ULS™), is based on the use of a platinum dye complex patented by KREATECH Biotechnology BV that forms a stable adduct with the N_7 position of guanine and, to a lesser extent, adenine bases in DNA, RNA, PNA, and oligonucleotides (Figure 1). The labeling reaction takes only 15 minutes and separation of the labeled nucleic acids from the unreacted ULS complex can be accomplished through the use of a simple spin-column procedure (Figure 2). The ULS method has been used to prepare labeled probes for dot, Southern and Northern blot analysis, RNA and DNA *in situ* hybridization, multicolor fluorescence *in situ* hybridization (mFISH), and comparative genome hybridization (CGH). Our ULYSIS® kits allow researchers to label DNA with our exceptionally bright and photostable fluorescent dyes, including the proprietary Alexa Fluor® dyes and Oregon Green® 488 dye (Table 1). Oregon Green® 488 dye can also be used as a hapten in combination with anti-fluorescein antibodies for fluorescence signal amplification or for chemiluminescent or colorimetric enzyme-linked detection methods.

The procedure below describes how to prepare fluorescent DNA hybridization probes optimized for chromosome *in situ* hybridization and dot blot hybridization. Suggestions for labeling RNA are also provided.

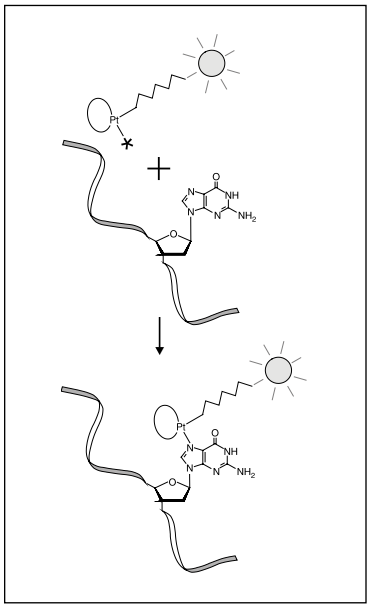

Figure 1. The ULS reagent in the ULYSIS® Nucleic Acid Labeling Kits reacts with the N7 of guanine residues to provide a stable coordination complex between the nucleic acid and the fluorophore label.

Materials

Contents

- **ULS labeling reagent** (Component A), 1 vial for 20 labelings or 5 vials, for 4 labelings each, in the case of Alexa Fluor® 488 ULS labeling reagent in kit U21650
- **Dimethylformamide (DMF, 50% solution in water)** or **Dimethylsulfoxide (DMSO)** (Component B), 200 µL
- **Labeling buffer** (Component C), 600 µL
- **Deoxyribonuclease I (DNase I)** (Component D), 100 µg
- **DNase I storage buffer** (Component E), 200 µL
- **10X DNase I reaction buffer** (Component F), 500 µL

Table 1. Spectral characteristics of the fluorescent dyes available in the ULYSIS® Nucleic Acid Labeling Kits.

	Fluorescent Dye	λ_{max} (nm) *	Em (nm) †	ε_{dye} ($cm^{-1}M^{-1}$) ‡	CF^{260} §	Spectrally Similar Dyes
U21650	Alexa Fluor® 488	492	520	62,000	0.30	Fluorescein (FITC)
U21651	Alexa Fluor® 532	525	550	82,300	0.24	Rhodamine 6G
U21652	Alexa Fluor® 546	555	570	104,000	0.21	Cy3, tetramethylrhodamine (TRITC)
U21653	Alexa Fluor® 568	576	600	93,000	0.45	Lissamine™ rhodamine B
U21654	Alexa Fluor® 594	588	615	80,400	0.43	Texas Red®
U21660	Alexa Fluor® 647	650	670	239,000	0.00	Cy5
U21656	Alexa Fluor® 660	660	690	107,000	0.00	Cy5 or Cy5.5
U21659	Oregon Green® 488	494	520	80,000	0.31	Fluorescein (FITC)
U21658	Pacific Blue™	410	455	36,000	0.15	SpectrumAqua™

* Absorbance maximum for the fluorophore; † Emission maximum for the fluorophore; ‡ Extinction coefficient for the dye; § Correction factor = A_{260} for the free dye / A_{max} for the free dye.

- **DNA from calf thymus** (Component G), 100 µL of a 0.1 mg/mL solution in TE buffer
- **Nuclease-free H_2O** (Component H), 5 mL

Sufficient materials are supplied for 20 labelings of 1 µg DNA each.

Storage and Handling

Upon receipt, store the kit at ≤–20°C in a desiccator, protected from light. When stored properly, the kit should be stable for at least six months.

Caution: No data are available addressing the mutagenicity or toxicity of the ULS labeling reagents. Because these reagents bind to nucleic acids, they should be handled with appropriate care. ULS labeling reagents should be disposed of safely and in accordance with applicable regulations. ULS labeling reagents can be removed from aqueous solutions by filtration through activated charcoal. The charcoal and adsorbed dye must then be disposed of in a safe and appropriate manner.

Materials Required but Not Provided

- Ethanol, absolute
- Sodium acetate, 3 M (pH 5.2)
- Spin column (see step 2.5)

Spectral Characteristics

For the best results, it is important to match the light source, excitation filters, and emission filters to the spectral characteristics of the dye. Please refer to Table 1 for this information.

Sample Preparation

The ULS labeling reagent will label double-stranded or single-stranded DNA of any length. However, DNA longer than about 1,000 bp may aggregate when labeled and precipitate out of solution, most likely due to hydrophobic interactions between closely spaced dye molecules. If such a probe is used for *in situ* hybridization, large aggregates may form over the sample, obscuring the signal. To produce optimally labeled probes, DNA longer than 1,000 bp should be fragmented by DNase I digestion prior to labeling as described below. Alternatively, it is possible to fragment the DNA by sonication or by digestion with a restriction enzyme that has a four-basepair recognition sequence. The fragmentation step is not needed for labeling DNA shorter than ~1,000 bp, or for labeling RNA samples (proceed to step 2.1).

DNase I Stock Solution

Centrifuge the vial of DNase I (Component D) briefly in a microcentrifuge to deposit the solids in the bottom of the tube. Make a 1 mg/mL DNase I stock solution by adding 100 µL of chilled DNase I storage buffer (Component E) to the vial of DNase I (Component D). MIX GENTLY by inversion to dissolve the DNase I. Do not vortex, as the DNase I is unusually sensitive to physical denaturation. Store the DNase I stock solution at ≤–20°C for up to six months.

DNase I Digestion Protocol

The following DNase I digestion protocol for 1 µg of DNA results in fragments ~100 bp to ~1,000 bp in length. This protocol has been successfully applied to a wide variety of DNA species and preparations with excellent results when subsequently labeled and used in FISH experiments. However, because DNase I preparations show some variation, we recommend that its activity be titrated before using it with your samples.

1.1 Prepare 200µL of 1X DNase I reaction buffer by adding 20 µL of 10X DNase I reaction buffer (Component F) to 180 µL of nuclease-free H_2O (Component H). Chill the solution on ice.

1.2 Immediately before use, make a DNase I working solution by diluting the DNase I stock solution into chilled 1X DNase I reaction buffer (from step 1.1). To find the optimal concentration of DNase I to use, test 1,650-, 2,500-, 5,000-, and 10,000-fold dilutions of DNase I using either the DNA from calf thymus (Component G) provided with the kit or a small portion of sample DNA. It is most convenient to perform the dilutions in two steps. First, dilute 1 µL of DNase I stock solution into 49 µL of 1X DNase I reaction buffer. Mix thoroughly by gently flicking the tube. Next, dilute 0.5, 1, 2, or 3 µL of the first dilution into 1X DNase I reaction buffer to a final volume of 100 µL. Mix thoroughly by gently flicking the tube. Leave DNase I working solutions on ice until ready to use. The working solutions are not stable and should be used only on the day prepared.

1.3 Add the following to a microfuge tube on ice in the order indicated, starting with the volume of nuclease-free H_2O (Component H) necessary to achieve a final volume of 25 µL. The following example is for 1 µg of DNA, from a 0.1 mg/mL solution:

- 9.5 µL nuclease-free H_2O (Component H)
- 2.5 µL 10X DNase I reaction buffer (Component F)
- 10 µL 0.1 mg/mL sample DNA in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)
- 3 µL DNase I working solution (from step 1.2)

1.4 Incubate at 37°C for 10 minutes. Stop the reaction by plunging the reaction tube into an ice bath.

1.5 To check the extent of DNase I digestion, take 2.5 µL from the reaction mixture, heat it at 65°C for 10 minutes and then analyze it by agarose gel electrophoresis. Confirm that the DNA has been digested to fragments between ~100 bp and ~1,000 bp in length.

Labeling Reaction

The optimal amount of ULS labeling reagent to use depends upon the application. Table 2 shows the optimal amount of ULS labeling reagent required to label 1 µg of DNA for hybridization to human metaphase chromosome spreads (FISH) or dot blots. To label more than 1 µg of DNA, we recommend using proportionally more ULS reagent and scaling up the reaction. Although we have not fully optimized the reaction for RNA labeling, preliminary experiments show good results using the same amounts of ULS reagent as for DNA labeling.

ULS Reagent Stock Solution

To prepare the ULS labeling reagent stock solutions, except for Alexa Fluor® 488 ULS reagent, add 100 µL of 50% DMF (Component B) or 100 µL of DMSO (Component B), depending upon the kit, to the vial containing the ULS reagent (Component A). Vortex mix until all of the ULS labeling reagent has dissolved and no particulate matter remains. In order to completely dissolve the ULS reagents, vigorous vortexing, followed by pipetting up and down, may be required. DO NOT HEAT the solution. ULS reagent stock solutions may be stored at 4°C for up to six months.

For the Alexa Fluor® 488 ULS reagent of product U21650, add 5 µL of DMSO (Component B) to one vial of the ULS reagent (Component A). Vortex mix until all of the ULS labeling reagent has dissolved and no particulate matter remains. The stock solution will be nearly colorless. The Alexa Fluor® 488 ULS reagent stock solution is stable for at least one month at 4°C.

Labeling Protocol

2.1 Precipitate 1 µg of DNA by adding 1/10 volume of 3 M sodium acetate (pH 5.2) and two volumes of absolute ethanol, freeze at –70°C for 30 minutes and then centrifuge for 15 minutes at 12K rpm. Wash the pellet with 70% ethanol and allow it to air dry. Resuspend the pellet in 20 µL of the labeling buffer (Component C). DO NOT use ammonium acetate for DNA precipitation, as the residual ammonium ions will interfere with the ULS labeling reaction. DO NOT use carrier DNA to assist in the ethanol precipitation, as this will also interfere with the ULS labeling reaction.

2.2 Denature the DNA (from step 2.1) at 95°C for 5 minutes and then snap cool on ice. Centrifuge the tube briefly to redeposit the sample to the bottom of the tube. Note: Denaturation is not required for labeling; however, it improves labeling efficiency by 20–40%.

2.3 Referring to Table 2, add the appropriate volume of ULS labeling reagent stock solution to the tube containing the denatured sample DNA. If necessary, add labeling buffer (Component C) to bring the final volume to 25 µL.

2.4 Incubate the reaction at 80°C for 15 minutes (for RNA, incubate at 90°C for 10 minutes). Stop the reaction by plunging the reaction tube into an ice bath. Centrifuge the tube briefly to redeposit the sample to the bottom of the tube.

2.5 The DNA must now be purified from the excess ULS labeling reagent. We recommend purifying the labeled DNA by using a gel filtration–based spin column, according to the manufacturer's protocol. For example, BioRad Micro Bio-Spin® P-30 or Princeton Separations Centri-Sep™ columns give good results. Avoid using silica-based separation techniques.

Calculating the Labeling Efficiency and Concentration of Nucleic Acid

The relative efficiency of a labeling reaction can be evaluated by calculating the approximate ratio of bases to dye molecules. This ratio can be determined by measuring the absorbance of the nucleic acid at 260 nm and the absorbance of the dye at its absorbance maximum (λ_{max}) and by using the Beer-Lambert law:

$$A = \varepsilon \times \text{path length (cm)} \times \text{concentration (M)},$$

where ε is the extinction coefficient in $cm^{-1}M^{-1}$. The absorbance measurements can also be used to determine the concentration of nucleic acid in the sample. Values needed for these calculations are found in Tables 1 and 3. Acceptable labeling ratios for ULS–labeled nucleic acids are listed in Table 2.

Table 2. Amount of ULS reagent solution required to label 1 µg of DNA for different applications.

ULS Labeling Reagent	Application		Acceptable Labeling Ratios *
	FISH	Dot Blots	
Pacific Blue™	5 µL	5 µL	53–72
Oregon Green® 488	3.5 µL	5 µL	24–51
Alexa Fluor® 488	1.0 µL	1.0 µL	43–67
Alexa Fluor® 532	1.5 µL	5 µL	7–16
Alexa Fluor® 546	5 µL	4 µL	44–89
Alexa Fluor® 568	5 µL	5 µL	43–79
Alexa Fluor® 594	5 µL	2.5 µL	40–98
Alexa Fluor® 647	5 µL	5 µL	30–40
Alexa Fluor® 660	5 µL	5 µL	25–33

* Base:dye ratio for the labeled nucleic acid. See *Calculating the Labeling Efficiency and Concentration of Nucleic Acid* in this document for instructions on how to determine this ratio.

Measuring the Base:Dye Ratio

3.1 Measure the absorbance of the nucleic acid–dye conjugate at 260 nm (A_{260}) and at the λ_{max} for the dye (A_{dye}). Measure the background absorbance at 260 nm and λ_{max}, using buffer alone, and subtract these numbers from the raw absorbance values for the sample. The λ_{max} values for the fluorophores used in the ULYSIS® kits given in Table 1.

- To perform these measurements, the nucleic acid–dye conjugate should be at a concentration of at least 5 µg/mL. Depending on the dye used and the degree of labeling, a higher concentration may be required.
- For most applications, it will be necessary to measure the absorbance of the entire sample using either a conventional spectrophotometer with a 100 or 200 µL cuvette or an absorbance microplate reader with a microplate.
- Use a cuvette or microplate that does not block UV light and that is clean and nuclease-free. Note that most plastic disposable cuvettes and microplates have significant absorption in the UV.

3.2 Correct for the contribution of the dye to the A_{260} reading. Most fluorescent dyes absorb light at 260 nm as well as at their λ_{max}. To obtain an accurate absorbance measurement for the nucleic acid, it is therefore necessary to account for the dye absorbance using a correction factor (CF_{260}). Use the CF_{260} values given in Table 1 in the following equation:

$$A_{base} = A_{260} - (A_{dye} \times CF_{260})$$

3.3 Calculate the ratio of bases to dye molecules using the following equation:

$$\text{base dye} = (A_{base} \times \varepsilon_{dye}) / (A_{dye} \times \varepsilon_{base})$$

Table 3. Average values for bases in different nucleic acids.

Nucleic Acid	ε_{base} $(cm)^{-1}(M)^{-1}$*	MW_{base} †
dsDNA	6,600	330
ssDNA	8,919	330
RNA	8,250	340

* Average extinction coefficient for a base; † Average molecular weight for a base (g/mol).

where ε_{dye} is the extinction coefficient for the fluorescent dye (found in Table 1) and ε_{base} is the average extinction coefficient for a base in double stranded DNA (dsDNA), long single-stranded DNA (ssDNA), or RNA (found in Table 3). Note that since the calculation is a ratio, the path length has canceled out of the equation.

Measuring the Concentration of Nucleic Acid

The absorbance values, A_{260} and A_{dye}, and the Beer-Lambert law may also be used to measure the concentration of nucleic acid in the sample ([N.A.]). In order to obtain an accurate measurement for a dye-labeled nucleic acid, a dye-corrected absorbance value (A_{base}) must be used, as explained in step 3.2. In addition, for concentration measurements, the path length (in cm) is required. If the path length of the cuvette or of the solution in a microplate well is unknown, consult the manufacturer. Follow steps 3.1 and 3.2 above and then use the following equation:

$$[\text{N.A.}]\ (\text{mg/mL}) = (A_{base} \times MW_{base}) / (\varepsilon_{base} \times \text{path length})$$

Product List *Current prices may be obtained from our website or from our Customer Service Department.*

Cat #	Product Name	Unit Size
U21650	ULYSIS® Alexa Fluor® 488 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21651	ULYSIS® Alexa Fluor® 532 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21652	ULYSIS® Alexa Fluor® 546 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21653	ULYSIS® Alexa Fluor® 568 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21654	ULYSIS® Alexa Fluor® 594 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21660	ULYSIS® Alexa Fluor® 647 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21656	ULYSIS® Alexa Fluor® 660 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21659	ULYSIS® Oregon Green® 488 Nucleic Acid Labeling Kit *20 labelings*	1 kit
U21658	ULYSIS® Pacific Blue™ Nucleic Acid Labeling Kit *20 labelings*	1 kit

Patent and Trademark Information

The use of labeled nucleic acid hybridization probes may be covered by patents belonging to third parties. Purchase of these kits does not provide a license to practice inventions covered by those patents.

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website—**probes.invitrogen.com**—for the most up-to-date information.

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