Methods for Enzymatic Incorporation of ChromaTide™ dUTPs

Introduction

ChromaTide™ dUTPs can be enzymatically incorporated into probes for use in many molecular biology applications. The use of ChromaTide™ nucleotides with different fluorescence emission profiles allows for multicolor applications. We have developed protocols for incorporating these nucleotides into hybridization probes using nick translation, random primer labeling, end-labeling with terminal deoxynucleotidyl transferase, reverse transcription or PCR amplification. Most of our ChromaTide™ dUTPs can be incorporated into probes using any of these protocols, although depending on your application, some further optimization of the reaction conditions may be necessary. The exceptions include BODIPY® 630/650 and BODIPY® 650/665 dUTPs, which are incompatible with PCR and reverse transcription. In addition, so far only Alexa Fluor® 488, Alexa Fluor® 532 and Alexa Fluor® 546 dUTPs have been tested and found suitable for incorporation by reverse transcriptase.

Nick Translation

Introduction

This protocol is suitable for all of the Alexa Fluor® ChromaTide™ dUTPs and for fluorescein ChromaTide™ dUTP. Modifications may be necessary for other ChromaTide™ dUTPs. Nick translation uses a combination of deoxyribonuclease I (DNase I) and DNA polymerase I to incorporate labeled nucleotides into DNA. DNase I nicks the double-stranded DNA template to create free 3′ hydroxyl ends. Then the polymerase activity of DNA polymerase I catalyzes the addition of a nucleotide residue to the 3′-hydroxyl terminus while the 5′-to-3′ exonuclease activity removes a nucleotide from the 5′-phosphoryl terminus of the nick. As these reactions continue, labeled and unlabeled nucleotides replace the excised nucleotides in the sequences, resulting in labeled fragments of various sizes and representing sequences from both strands of the template. The probe must be denatured into single-stranded fragments before hybridization.

Materials Required

- ChromaTide dUTP, 1 mM solution
- ~1 µg of template DNA
- d(GAC) mixture containing 0.5 mM each of dGTP, dATP, dCTP
- 0.1 mM dTTP
- DNase I (Roche #104159)
- 10X nick translation buffer (0.5 M Tris-HCl, 50 mM $MgCl_2$, 0.5 mg/mL nuclease-free BSA (Roche #711454), pH 7.8)
- DNase I storage buffer (20 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 100 µg/mL nuclease-free BSA, 50% glycerol, pH 7.6)

Preparing the Template

For optimal results, use linearized DNA purified by phenol/chloroform extraction and ethanol preciptiation. Dilute the template to about 1 µg/µL in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

Labeling Reaction

1.1 Prepare a DNase I stock solution by dissolving 1 mg of DNase I in 1 mL of DNase I storage buffer. Store at –20°C.

1.2 Make a fresh working dilution of DNase I by diluting 1 µL of the 1 mg/mL DNase I stock solution (prepared in step 1.1) into 1 mL of the 1X nick translation buffer. Leave on ice.

1.3 Add the following to a microfuge tube on ice in the order indicated:

- 22 µL dH_2O
- 5 µL 10X nick translation buffer
- 5 µL 0.1 M DTT
- 4 µL d(GAC)TP mixture (0.5 mM dGTP, 0.5 mM dATP, 0.5 mM dCTP)
- 5 µL 0.1 mM dTTP
- 1.5 µL 1 mM ChromaTide dUTP
- 1 µL ~1 µg/µL DNA template
- 5 µL DNase I (1 µg/mL solution prepared in step 1.3)
- 1.5 µL DNA polymerase I (Promega catalog # M2051, 10 U/µL)

1.4 Incubate at 15°C for 2 hours. Bring to a final volume of 100 µL with 50 µL dH_2O nuclease-free water and proceed to purification.

Random Primer Labeling

Introduction

To make labeled probes using the random primer–labeling method, random-sequence primers between six and nine bases long are hybridized to denatured template DNA. Using the template as a guide, the Klenow fragment of DNA polymerase I extends the primer to synthesize a new DNA strand, incorporating some of the fluorophore-labeled dUTPs in place of dTTPs. The resulting probe consists of fluorophore-labeled fragments of various sizes, representing sequences from both strands of the template. The probe must be denatured into single-stranded fragments before hybridization.

Materials Required

- ChromaTide dUTP, 1 mM solution
- 10 ng to 3 µg template DNA (we typically use 750 ng)
- Klenow fragment of DNA polymerase I

- dATP, dCTP, dGTP, dTTP, 10 mM solutions
- Random sequence primers (such as Roche catalog #1277081)
- 10X Klenow buffer: 0.5 M Tris-HCl, pH 7.5, 0.1 M $MgCl_2$, 10 mM DTT, 0.5 mg/mL BSA
- EDTA, 0.2 M, pH 8.0

Preparing the Template

For optimal results, use linearized DNA purified by phenol/chloroform extraction and ethanol precipitation. Dilute the template to about 0.1 µg/µL and incubate at 95°C for 5 minutes to denature. Rapidly cool the denatured template in an ice-water slurry.

Preparing a 10X Nucleotide Mixture

Prepare a 10X dNTP mixture, having the final concentrations:

- 1 mM dATP
- 1 mM dCTP
- 1 mM dGTP
- 0.65 mM dTTP
- 0.35 mM ChromaTide™ dUTP

An excess of this mixture may be prepared and stored frozen at –20°C or –80°C for up to 6 months.

Labeling Reaction

2.1 Make up the following mixture, adding the enzyme last:

- ~8 µL heat-denatured template DNA, prepared above
- 2 µL 10X Klenow buffer
- 2 µL 10X dNTP mixture, prepared above
- 2 µL random sequence primers (final concentration = 200 µg/mL)
- dH_2O to bring the final volume (including enzyme) to 20 µL
- 2 units Klenow enzyme (about 1 µL)

Mix gently and thoroughly, and spin in a microcentrifuge briefly to collect the reaction into the bottom of the tube.

2.2 Incubate reactions at 37°C for 2–8 hours. Increasing the length of the incubation generally increases the amount of reaction product obtained. We typically incubate the reaction for about 6 hours.

2.3 Stop the reaction by adding 2 µL of 0.2 M EDTA, pH 8.0 and mixing. Store at 4°C until ready to use.

Terminal Transferase Labeling

Introduction

Terminal deoxynucleotidyl transferase (TdT) adds nucleotides to the 3′ terminus of DNA molecules. This is the preferred method for labeling oligonucleotide probes. The reaction can be performed in two ways. Short tails are obtained by including only the ChromaTide™ dUTP in the reaction mixture; long tails can be obtained by including both ChromaTide™ dUTP and unlabeled nucleotide (e.g., dATP). The use of labeled and unlabeled nucleotide for longer tails may improve incorporation rates and decrease self-quenching of the fluorophores, leading to more brightly fluorescent oligonucleotide probes. The enzyme itself does not require the presence of ATP as an energy source. Please note that this is not the same protocol that would be used for the TUNEL assay. For protocols on using fluorescent nucleotides in the TUNEL assay, see Exp Cell Res 222, 28 (1996).

Materials Required

- ChromaTide dUTP, 1 mM solution
- Oligonucleotide, 100 pmol
- $CoCl_2$, 25 mM
- dATP, 10 mM (for longer tails, optional)
- 5X Terminal transferase reaction buffer, provided with the enzyme or use: 1 M potassium cacodylate, 125 mM Tris-HCl, 1.25 mg/mL bovine serum albumin, pH 6.5 at 25°C
- Terminal deoxynucleotidyl transferase
- EDTA, 0.2 M, pH 8.0

Preparing the Template

We recommend purifying oligonucleotides with phenol/chloroform extraction followed by ethanol precipitation. You may find that further purification is necessary in order to get good hybridization results in your application. If you want to label a smaller or larger amount of the oligonucleotide, follow the instructions included with the enzyme to calculate the appropriate amount of enzyme to use in the reaction.

Labeling Reaction

3.1 Make up the following mixture, adding the enzyme last:

- 100 pmol oligonucleotide
- 4 µL 5X terminal transferase reaction buffer
- 4 µL 25 mM $CoCl_2$
- 1 µL 1 mM ChromaTide dUTP
- 1 µL 10 mM dATP (optional)
- dH_2O to bring the final volume (including enzyme) to 20 µL
- 50 units terminal deoxynucleotidyl transferase enzyme (~1 µL)

Mix gently and thoroughly, and spin in a microfuge briefly to collect the reaction into the bottom of the tube.

3.2 Incubate at 37°C for 15 minutes.

3.3 Stop the reaction by adding 2 µL of 0.2 M EDTA, pH 8.0 and mixing. Store at 4°C until ready to use.

Reverse Transcription

Introduction

Reverse transcriptase can be used to produce fluorophore-labeled single-stranded DNA from an RNA template using appropriate primers. The following method was optimized using 3 µg of total bacterial RNA, random hexamer primers, Superscript™ II reverse transcriptase (Life Technologies, Inc.) and Alexa Fluor® 546 dUTP for use in microarray experiments. Random hexamer primers were found to produce the best labeling results for this RNA sample in this type of experiment. Modifications may be required if using other types of RNA samples, primers or enzymes. The most useful parameter to modify is the ratio of labeled ChromaTide™ dUTP to unlabeled dTTP.

Materials Required

- ChromaTide™ dUTP, 1 mM solution
- 3 µg of total RNA
- Reverse transcriptase

- d(GACT) mixture containing 5 mM each of dGTP, dATP, dCTP and 2.27 mM of dTTP
- Primers, ~2 mg/mL
- 5X first-strand buffer for reverse transcriptase (250 mM Tris, 375 mM KCl, 15 mM $MgCl_2$, pH 8.3)
- 100 mM DTT

Labeling Reaction

4.1 Add dH_2O to the 3 µg sample of RNA to bring the final volume to 11 µL.

4.2 Add 2 µL of primers (~2 mg/mL) and mix thoroughly.

4.3 Heat the template and primer mixture to 98°C for 10 minutes and then snap cool on ice.

4.4 To the tube containing the denatured template and primers, add:
- 5 µL 5X first-strand buffer
- 2.5 µL 100 mM DTT
- 2.3 µL d(GACT)
- 1.5 µL ChromaTide dUTP (1 mM solution)

Mix well and then add:
- 2 µL reverse transcriptase

4.5 Incubate the reaction at 25°C for 10 minutes. Then, incubate the reaction at 42°C for 90 minutes.

4.6 The reaction may be stored at 4°C or, for longer term, at –20°C.

Note that this protocol does not require digestion of the RNA. However, it will be necessary to digest the RNA in order to obtain an accurate measurement of the base:dye ratio. We strongly suggest measuring the base:dye ratio, at least for the first time the labeling reaction is performed. We suggest using RNase H digestion or denaturation followed by RNase A digestion. DO NOT use base hydrolysis of the RNA, as this will destroy the fluorophore. Bring the mixture to a final volume of 100 µL with nuclease-free H_2O and proceed to *Purification of RT-Labeled DNA*.

Purification of RT-Labeled DNA

Purify the labeled DNA using a Qiagen QiaQuick™ PCR Purification Kit using the manufacturer's protocol, followed by ethanol precipitation.

PCR Labeling

Introduction

In PCR labeling, short oligonucleotides complementary to different regions of a template sequence serve as primers for a thermostable DNA polymerase. Cycles of denaturing, annealing and extension temperatures allow amplification of the DNA between the two primers. When a fluorescent ChromaTide™ dUTP is added to this reaction, a fluorophore-labeled double-stranded DNA probe is generated. We tested the following protocols using an expression vector containing a mouse actin cDNA and primers to the T3 promoter and T7 promoter regions of the vector. You may find it necessary to modify this protocol for your template and primers.

Materials Required

- ChromaTide dUTP, 1 mM solution
- 1 ng template, about 1 ng/µL
- *Taq* DNA polymerase
- dATP, dCTP, dGTP, 10 mM solutions
- dTTP, 2 mM solution
- Primers, 5 µM each
- 10X PCR buffer. Use the 10X buffer solution supplied with the enzyme or 100 mM Tris-HCl, pH 8.3, 500 mM KCl
- $MgCl_2$, 25 mM

Preparing the Template

For optimal amplification, use DNA purified by phenol/chloroform extraction and ethanol precipitation. For amplification with BODIPY® ChromaTide™ dUTPs, avoid ammonium acetate in the ethanol precipitation when preparing template and primers. The BODIPY® dyes may become unstable in the presence of heat and ammonia.

Labeling Reaction

The following reaction conditions are suitable for any of our ChromaTide™ dUTPs, except for the BODIPY® 630/650 and the BODIPY® 650/665 ChromaTide™ dUTPs, neither of which appear to be incorporated into PCR products. Modifications to this method are required for ChromaTide™ Alexa Fluor® 532, BODIPY® TR and BODIPY® FL dUTPs, as described in *Special Modifications for PCR Reactions*. It may also be necessary to increase the ratio of unlabeled to labeled nucleotide, as described in step 5.3 under *Troubleshooting PCR Reactions* (below).

5.1 Make up the following mixture, adding the enzyme last. If multiple reactions are being run simultaneously, all of the shared components, with the exception of the ChromaTide™ dUTP and the enzyme, are typically made up into a mixture and aliquoted into reaction tubes.
- 5 µL 10X PCR buffer
- 3 µL 25 mM $MgCl_2$
- 1 µL 1 ng/µL template DNA
- 2 µL primer mix, 5 µM each
- 1 µL 10 mM dATP
- 1 µL 10 mM dCTP
- 1 µL 10 mM dGTP
- 3.75 µL 2 mM dTTP
- 2.5 µL 1 mM ChromaTide™ dUTP
- dH_2O to bring the final volume (including enzyme) to 50 µL
- 2.5 units *Taq* DNA polymerase (~0.5 µL)

Mix gently and thoroughly, and spin in a microfuge briefly to collect the reaction into the bottom of the tube.

5.2 Cycle the reaction under the following conditions:
- 1 cycle:
  5 min at 94°C
  5 min at 55°C
- 30 cycles:
  2 min at 72°C
  1 min at 94°C
  1 min at 55°C
- 1 cycle:
  5 min at 72°C

5.3 Hold at 4°C until ready to use.

Special Modifications for PCR Reactions

The following modifications are necessary in order to get good incorporation of certain ChromaTide™ dUTPs into PCR amplification products. To obtain clean PCR products with these nucleotides, we have modified four variables in the reactions: addition of DMSO to a final concentration of 10%, addition of Tween® 20 to a final concentration of 0.1%, titration of the labeled nucleotide and unlabeled nucleotide ratios, and post-PCR purification. The conclusions of these experiments are listed below by nucleotide:

- **ChromaTide™ Alexa Fluor® 532-5-dUTP**: This nucleotide is incorporated well into PCR products but requires a post-reaction purification step to obtain clean product. See *Purifying the Probe* (below).
- **ChromaTide™ BODIPY® TR-14- dUTP**: Incorporation requires a ratio of 20 µM ChromaTide dUTP to 180 µM unlabeled TTP and the addition of 0.1% Tween 20.
- **ChromaTide™ BODIPY® FL-14-dUTP**: Incorporation requires a ratio of 20 µM ChromaTide dUTP to 180 µM unlabeled TTP.

Troubleshooting PCR Reactions

6.1 Run 20% of the reaction on an agarose gel and view on a UV transilluminator. If you do not see a fluorophore-labeled product of the appropriate length, post-stain the gel with SYBR® Gold stain (or ethidium bromide) to determine if there is any unlabeled reaction product. This will tell you whether the PCR amplification itself is working.

6.2 If you determine that the PCR amplification is working, but there is no incorporation of the ChromaTide™ dUTP into the product, you may need to optimize the reaction conditions for your template and primers. We recommend adding in variable amounts of DMSO and/or Tween® 20 in the reaction, as described in *Special Modifications for PCR Reactions* (above).

6.3 For certain templates, the ratio of labeled dUTP to unlabeled dTTP in the reaction may have to be altered. For our typical template, we use a 1:3 molar ratio of labeled to unlabeled nucleotide. If the products of the reaction are too short and variable in length, the polymerase may be stalling after addition of the ChromaTide™ dUTP. In these cases, full length probes can be obtained by using lower ratios of labeled to unlabeled nucleotide.

6.4 The extension time should be optimized for the probe. Longer probes may require longer extension times.

Purifying the Probe (optional)

Although labeled products can be visualized directly on electrophoretic gels, it may be necessary to remove unincorporated nucleotides from the reaction mixture prior to use of the labeled products in subsequent experiments. The procedure described below has been used successfully with all of the labeled nucleotides. Ethanol precipitation is not recommended as an alternative step because with some conjugates, such as the Alexa Fluor® dye conjugates, it may not efficiently remove the nucleotides from the reaction mixtures. Other spin column methods may also work well.

7.1 After the enzymatic labeling reaction is complete, overlay the reaction onto a pre-wet Centri-Sep™ spin column (Princeton Separations, Inc., Adelphia, N.J., catalog # CS-900). Small amounts of mineral oil from the PCR reaction do not seem to inhibit or interfere with the purification, but avoid transferring large amounts of oil as it may interfere with later hybridization steps.

7.2 Follow the manufacturer's instructions in centrifuging the column. After spinning the column, most of the color will remain on the column, and the eluate containing the labeled probe will have very little color because most of the fluorophore-labeled dUTP will not be incorporated into the probe. Recoveries are typically ~80% or better, based on visual estimates from agarose gel analysis, before and after purification.

7.3 Reaction products can be concentrated using Microcon™ Microconcentrators (Amicon, Inc., Beverly, MA, catalog #42412) following the manufacturer's instructions. Yields from this step are typically about 80%.

7.4 You may analyze ~10% of the reaction on a 1% agarose (random primer or PCR products) or a 20% polyacrylamide gel (19:1) in 7 M urea (labeled oligonucleotides). The fluorescent products can be viewed on a typical UV transilluminator and photographed using Polaroid® 667 black-and-white print film and an appropriate filter, such as the SYBR® Green/Gold photographic filter (catalog # S-7569). To assess the purity of the probe, first view the unstained gel, then stain the gel with a sensitive gel stain like SYBR® Green stain or SYBR® Gold stain to detect unlabeled DNA species. The BODIPY® ChromaTide™ dUTPs cause PCR products to migrate at slightly slower rates. Random primer–labeled products will run as a smear on gels.

Measuring the Base:Dye Ratio for Fluorophore-Labeled Nucleic Acids

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:

$$A = \epsilon \times \text{path length} \times \text{concentration},$$

where ϵ is the extinction coefficient in $cm^{-1}M^{-1}$. Values needed for the calculations are found in Tables 1 and 2.

Measuring the Base:Dye Ratio

8.1 Measure the absorbance of the DNA–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 are given in Table 1.

Table 1. Absorption characteristics for fluorescent dyes.

Fluorescent Dye	λ_{max} (nm)*	ϵ_{dye} (cm^{-1}M^{-1})†	CF$_{260}$‡
Alexa Fluor 488	492	72,000	0.30
Alexa Fluor 532	525	82,300	0.24
Alexa Fluor 546	555	104,000	0.21
Alexa Fluor 568	576	92,300	0.46
Alexa Fluor 594	588	80,400	0.43
BODIPY 630/650	632	100,000	0.09
BODIPY 650/665	651	101,300	0.07
BODIPY FL	504	82,000	0.00
BODIPY TMR	535	97,500	0.15
BODIPY TR	588	85,000	0.11
Cascade Blue	400	27,000	0.18
Fluorescein	494	80,000	0.32
Oregon Green 488	494	80,000	0.31
Rhodamine Green	500	78,000	0.24
Tetramethylrhodamine	550	100,000	0.27
Texas Red	593	85,000	0.23

* Absorbance maximum for the fluorophore † Extinction coefficient for the dye ‡ Correction Factor = A260 for the Fluor dye / Amax for the Fluor dye

- To perform these measurements, the DNA–dye conjugate should be at a concentration of at least 5 µg/mL. Depending on the dye you use 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 plate 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.

Table 2. Average extinction coefficients for a base in different nucleic acids.

Nucleic Acid	ϵ_{base} (cm^{-1}M^{-1})*	MW$_{base}$†
dsDNA	6600	330
ssDNA	8010	330
oligonucleotide	10,000	330

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

8.2 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:

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

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

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

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 oligonucleotides (found in Table 2). Note that because that you are calculating 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 your sample ([N.A.]). 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 8.2. 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 8.1 and 8.2 above and then use the following equation:

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

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 Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

Molecular Probes, Inc.
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504
Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com
Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228
Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419
Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl
Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

For research use only. The dUTP and UTP nucleotides are NEN-brand products that are distributed and sold under an agreement between ENZO DIAGNOSTICS, INC. and PERKINELMER LIFE SCIENCES, INC. (previously NEN LIFE SCIENCES, INC.) for research purposes only by the end-user in the research market and are not intended for diagnostic or therapeutic use. Purchase does not include or carry any right or license to use, develop or otherwise exploit this product commercially. Any commercial use, development or exploitation of these products without the express prior written authorization of ENZO DIAGNOSTICS, INC. and PERKINELMER LIFE SCIENCES, INC. is strictly prohibited. These products or the use of these products may be covered by one or more Enzo patents, including the following: U.S. Patent No. 5,328,824 and DK 164 407 8.