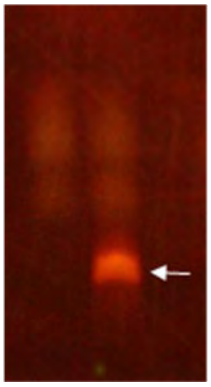
PRODUCT INFORMATION SHEET



OiGlo™

UNIVERSAL NUCLEIC ACID LABELING KIT

Product M1600 Series

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OliGlo™ Universal Nucleic Acid Labeling Kit
(Product M1600 Series)

NOTE: This kit series provides a robust method for labeling oligonucleotides, RNA or DNA samples using *in situ* prepared reactive compounds. The reactive compounds create stable phosphodiester and phosphotriester linkages between reporter molecules (fluorescent, chemiluminescent, receptor ligand or chromogenic moieties) and internal or terminal phosphate moieties of nucleotides. For more information about these techniques, please visit our website at www.markergene.com or contact our technical assistance department at techservice@markergene.com.

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I. OVERVIEW

A number of agents have been described for labeling nucleic acids to facilitate detection of target DNA or RNA sequences. Suitable labels may provide signals detectable by fluorescence, radioactivity, colorimetry, X-ray diffraction or absorption, magnetism or enzymatic activity. It is essential that the labeling method not perturb base-pairing hybridization critical for preserving assay specificity. Nevertheless, several common methods including labeling by enzymatic incorporation can often lead to interference with the subsequent hybridization detection step, because current fluorescent labels are attached to the base (purine, pyrimidine) portion of the nucleotides where base-pairing and hybridization occurs. In addition, enzymatic labeling methods make use of additional enzymatic steps which require precise calibration to achieve a reproducible labeling yield. Moreover, because the enzymes used depend on the target type (DNA or RNA) and sequence, sequence perturbation is often observed.

To remedy this, methods of direct labeling have been used with varying degrees of success. Platinum-based ("ULYSIS") or guanosine reactive (Mirus) direct labeling compounds have been developed, but also label through the base portion of DNA/RNA sequences. Direct labeling through phosphodiester and phosphotriester linkages on the DNA or RNA backbone provides the additional advantage of reducing the perturbation of base-pairing hybridization. Our OliGlo™ kits utilize a direct labeling methodology through reaction with the phosphate groups (terminal and backbone) on oligonucleotides, DNA or RNA. The active labeling reagents are prepared *in situ* from stable precursor molecules derived from a variety of highly fluorescent dyes and other detection labels, allowing the highly reactive labels to function at optimum efficiency for each

sample. The OliGlo™ kits allow molecular biologists and clinical researchers to label or monitor genomic DNA or RNA samples, nucleotides or oligonucleotides for easy detection and quantification.

The supplied standard labeling protocol will yield labeling efficiency of approximately 10 to 100 labels per kilobase of nucleic acid depending on the properties of the different labeling dyes and purity of the sample (Table 1). We found that this labeling density is sufficient for most applications. Should there be a need for adjusting labeling efficiency, the end user can simply modify the ratio of labeling dye to nucleic acid as well as incubation times for the labeling reaction as necessary.

Table 1: Excitation and emission wavelength for fluorescent OliGlo™ labeling reagents.

Fluorophore	EX (nm)	EM (nm)
TAMRA	552	582
Cy3	548	567
Cy5	649	670
Fluorescein	488	520

II. MATERIALS

- OliGlo™ Universal labeling dye precursor (dried pellet)
- Anhydrous DMF (for reconstituting the labeling reagent precursor)
- Activating Resin
- 3M NaOAc, pH5.2 (for DNA/RNA precipitation)
- Denaturation buffer D
- Renaturation buffer R
- TE buffer (for reconstituting the labeled DNA/RNA samples).
- Absolute Ethanol (for precipitating DNA or RNA samples – Not Provided)

If required, additional detection reagents, including conjugated antibodies, conjugated streptavidin and chemiluminescent substrates can be obtained from Marker Gene Technologies, Inc. or from a variety of commercial sources.

Storage and Handling. Store the OliGlo™ dye precursor and activating resin frozen (-20°C). Once reconstituted in DMF or activated with activating resin, the reagent should be capped tightly and stored desiccated at -20°C. Avoid exposure to moisture and light. In case of contact with skin or eyes, wash thoroughly with soap and cold water. Un-reconstituted and un-converted labeling precursors are stable for at least 6 months upon receipt. Once reconstituted and converted, the activated labeling reagent should be used within 20 days.

III.LABELING PROTOCOL

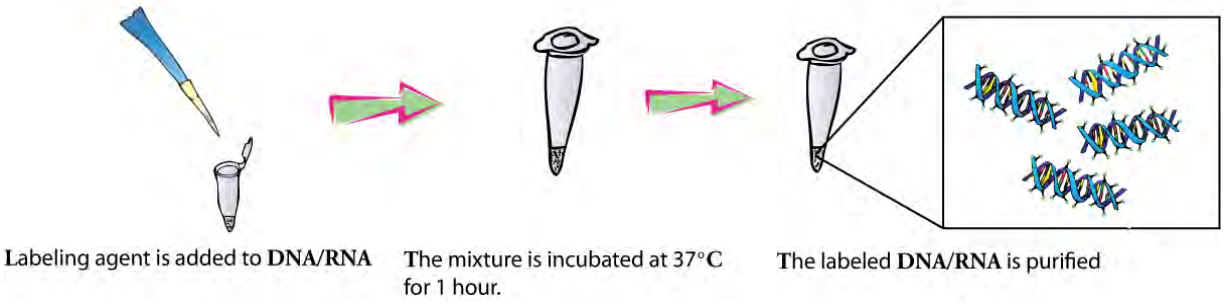

Figure 1. Overview of labeling protocol

Nucleic acid preparation:

1. To ensure efficient labeling, the nucleic acid should be dissolved in RNase and DNase free water. Avoid EDTA, phosphate or acetate-containing buffers, since these will cause side reactions with the labeling reagent. The A260/A280 of the purified nucleic acid should be in the range of 1.8-2.2.

NOTE: If your DNA or RNA sample is in another buffer, purify first by ethanol precipitation followed by reconstitution in RNase and DNase free water. Protein impurities affecting the A260/A280 ratio can be removed by phenol:chloroform extraction prior to ethanol precipitation.

Preparation of active labeling dye:

1. Prior to each use, warm the vials containing the labeling dye precursor, resin and DMF to room temperature. Spin briefly before opening.

2. Add 150µL of DMF to the dried pellet to resuspend at 5 mM concentration. If using a trial size kit, add 40 µL of DMF. To ensure complete resuspension, you can add one-half volume (½V) of DMF to the pellet, vortex briefly and transfer to the tube containing the activating resin. Add another ½V of DMF to the labeling dye tube, vortex briefly and transfer again to the resin tube.

3. Mix resin and resuspended dye precursor thoroughly by vortexing. Then store at room temperature in the dark for at least 16 hours before labeling nucleic acids.

Labeling reaction:

Note: Spin the tube containing resin and dye mixture briefly to completely pellet the resin. Avoid pipetting up the resin when aspirating the active labeling dye.

4. Prepare the labeling reaction according to the example shown below. Use DNase and RNase-free water, and add labeling reagent last.

DNase and RNase-free H_2O	40µL
1mg/ml nucleic acid	5µL
Activated labeling reagent soln. (5 mM in DMF)	5µL
Total volume:	50µL

For less water-soluble labeling dyes, such as for Biotin-X labeling, more DMF is included so that the reaction takes place in 1:1 of H_2O:DMF.

DNase and RNase-free H_2O	20µL
1mg/ml nucleic acid	5µL
DMF	20µL
Activated labeling reagent soln. (5 mM in DMF)	5µL
Total volume:	50µL

5. Incubate the reaction at 37^{o}C for 1 hour. Do a quick spin at the end of labeling reaction.

Purify the labeled sample by ethanol precipitation:

6. Bring the total volume to 200µl with DNase and RNase-free water.

7. For DNA, add 20µL (1/10 of the total volume) 3M NaOAc, pH5.2 and 400 µL (2V) 100% ethanol.
   For RNA, add 20µL (1/10 of the total volume) 3M NaOAc, pH5.2 and 500µL (2.5V) 100% ethanol.

8. Mix well and place at -20^{o}C or lower temperature for at least 30 minutes.

9. Centrifuge for 15 minutes to pellet the labeled nucleic acid. Aspirate the ethanol, careful not to disturb the pellet.

10. Gently wash the pellet once with 500-1000µl 70% ethanol prepared with DNase and RNase-free water. Then centrifuge for an additional 10 minutes.

11. Remove all traces of ethanol with a micropipetter. Do not allow the sample to air dry extensively, as the pellet may become difficult to resuspend.

12. Resuspend the pellet in an appropriate volume of TE buffer. Store at -20^{o}C or proceed with application as required.

Notes:
This example labels 5µg of nucleic acid at a 1:1 (v:w) ratio of labeling reagent to nucleic acid. This ratio will result in labeling efficiencies that are appropriate for most applications. Should it be necessary to increase or decrease the density of labels in nucleic acids, simply modify the ratio of labeling reagent to nucleic acid or incubate at 37^{o}C for longer than 1 hour. In addition, the labeling reaction may be scaled up or down, depending on the amount of nucleic acid to be labeled.

IV. DETERMINING THE INCORPORATION OF LABELS IN THE NUCLEIC ACID SAMPLE:

Fluorescence dye incorporation can be measured using one of the following methods:

1. Measure spectrophotometric absorbance using the λmax of the dye (see dye table) as well as the nucleic acid concentration (at 260 nm) using a spectrometer such as the NanoDrop 2000 (Thermo Scientific) or similar instrument. Normalize the dye λmax to nucleic acid ratio of concentrations and compare with un-labeled samples.

2. Run the labeled nucleic acid sample on agarose gel and observe under UV illuminator to identify the labeled bands, and further confirm the bands by post-gel staining with Ethidium Bromide (0.5µg/ml in water) (see Figure 2 below for an example).

3. Spot serial dilutions of purified labeled sample onto a glass slide and view under a fluorescent microscope. Compare with unlabeled samples.

Biotin incorporation can be measured in the following methods:

1. Incubate labeled nucleic acid with equal amount of streptavidin at room temperature for 15 minutes and then analyze the reaction solution using

agarose gel electrophoresis. A shift of the nucleic acid band (slower migration) with the presence of streptavidin indicates the successful incorporation of the biotin moiety (see Figure 3 below).

2. Fix dilutions of labeled sample to a membrane, then detect with fluorescent-, alkaline phosphatase- (color) or HRP- (chemiluminescence) conjugated streptavidin.

Application Notes:

This labeling reagent can be used for non-radioactive hybridization, Fluorescent *In Situ* Hybridization and Microarray analysis. Please see our other kits (M1620 and M1640 series) for more details about these analyses.

M1600 SERIES KIT CONTENTS			
KIT COMPONENT	**TRIAL SIZE**	**STANDARD KIT**	**CAP COLOR**
REAGENTS			
OliGlo™ labeling dye precursor	dried pellet	dried pellet	amber glass vial
DMF	50µL	200 µL	black
Activating Resin	dried powder	dried powder	white
Denaturation buffer D	50 µL	250 µL	blue
Renaturation buffer R	50 µL	250 µL	yellow
3M NaOAc, pH5.2	100 µL	500 µL	orange
TE buffer	100 µL	500 µL	green
DOCUMENTATION			
MSDS	1	1	

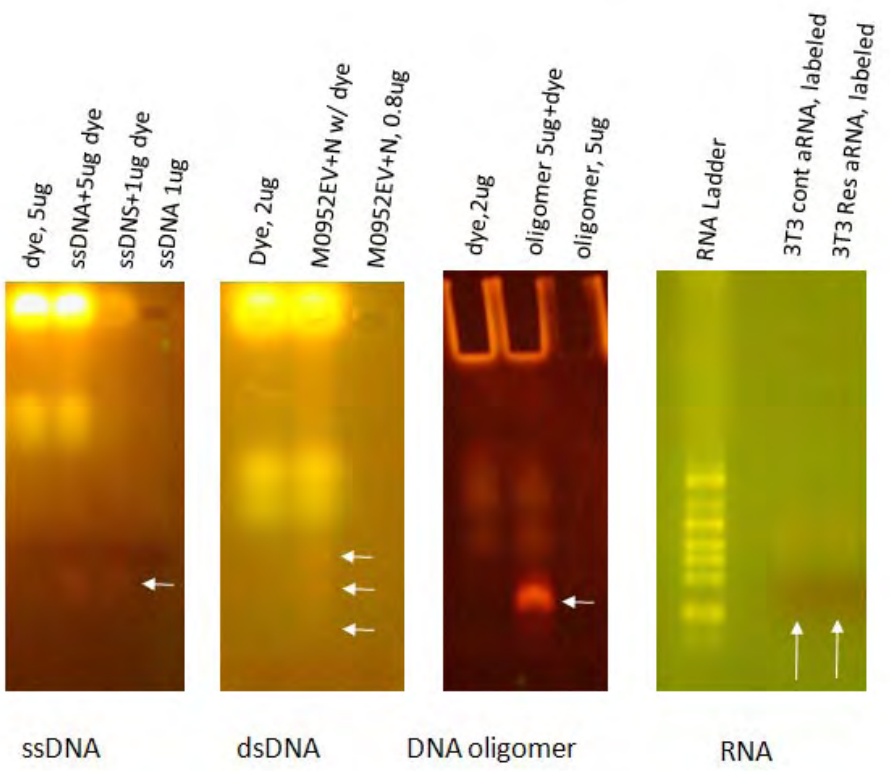

Figure 2: OliGlo™ TAMRA Universal Nucleic Acid Labeling Kit (M1600/M1601) is able to label ssDNA, dsDNA, oligonucleotides and amplified RNA: labeled products were applied to agarose gel electrophoresis. Fluorescent bands were further confirmed to be relevant nucleotides by staining with ethidium bromide (0.5μg/mL in water)

Table 2: Incorporation of labeling dye in DNA oligomers.

Dye	Oligo conc ng/ul	Maximum abs	OD	Dye Ext. Coeff	Dye conc moles/L	Oligo conc moles/L	DOS (dyes/1000bp)
TAMRA	289.6	552nm	0.029	95000	3.05E-07	2.70E-05	11
Cy3	235.6	546nm	0.319	130000	2.45E-06	2.20E-05	112

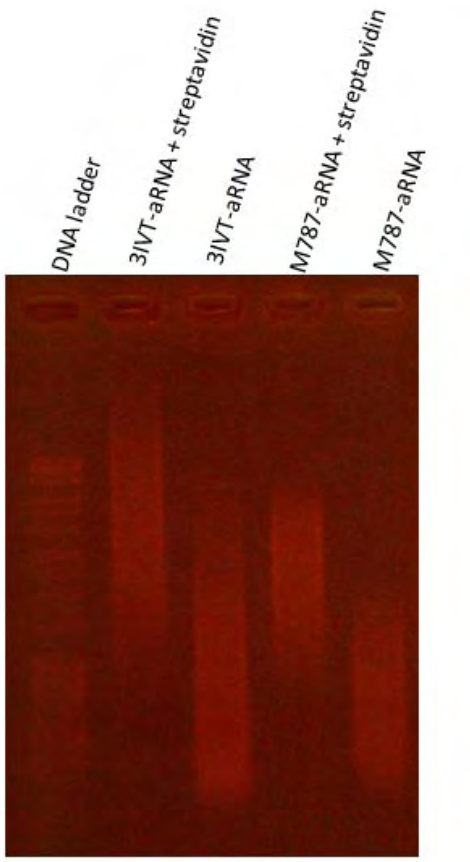

Figure 3: OliGlo™ Biotin Nucleic Acid Labeling Kit (M1604/1605) directly labels amplified RNA (aRNA) revealed by Streptavidin supershift: biotin labeled aRNAs were generated either with Affymetrix GeneChip 3'IVT express kit or by direct labeling with our OliGlo™ Biotin Nucleic Acid Labeling Kit, incubated with Streptavidin and applied to agarose gel electrophoresis followed by ethidium bromide staining.

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