Warning:
This product is for research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Introduction

The Oyster®-Array DNA Kit was especially developed for high efficiency labelling of DNA and RNA samples, which have been modified with amino functionalized nucleotides, e.g., aminoallyl-dUTP, aminoallyl-UTP, etc. This labelling procedure, which is known as secondary or two step labelling, comprises the incorporation of amino-modified nucleotides, which gives more uniform incorporation rates as compared to the direct labelling, using fluorescently labelled nucleotides. After purification of the amino-modified probe, the amino reactive fluorescent dye is covalently bound with high labelling efficiency, regardless of the type of dye chosen.

Formulation

The kit comprises 2 x 12 (12 for each dye) individually packed with desiccant and sealed micro-tubes of our Oyster® 550 and 650 fluorescent dyes, activated as tetrafluorphenyl-esters (TFP). In contrast to the established N-hydroxy-succinimidyl-ester (NHS), TFP esters are characterized by a higher coupling efficiency in aqueous environment. Each tube contains 40,000 pmol of the dye, sufficient to label up to 1 µg amino-modified cDNA/RNA.

Storage

Keep the micro-tubes at 4°C or lower before opening. Protect from light during storage. Each micro-tube is individually packed with desiccant and sealed. Prior to your experiments, let the appropriate number of packages allow reaching room temperature before opening, in order to prevent moisture contamination. Under these conditions the product should be stable for at least 6 months.

Applications

Synthesis of amino-modified cDNA

For synthesis of amino modified cDNA follow the instructions given by the manufacturer of the reverse transcriptase used.

Purification of the amino-modified cDNA

Column purification or ethanol precipitation has both been approved for purification of amino-modified cDNA.

A) The purification of the labeled oligonucleotide may be achieved simplest by commercial available quick separation columns (e.g., QIAquick™ PCR Purification Kit) following the instructions given by the supplier.

B) As an alternative, the cDNA may be purified by ethanol precipitation.

In either case, the purification has to be repeated several times, as traces of free amino-allyl dUTP will consume activated dye and thus reduce the labelling efficiency with your cDNA.

Labeling with Oyster®-550 or 650 TFP

After dissolving the amino-modiefied cDNA in a small volume (2-10 µl) of bidest. nuclease free water, 5 µl of coupling buffer is added. Best results were achieved using 0.1 M sodium bicarbonate buffer, pH 8.0.

Allow the sealed envelope with Oyster® dyes to reach room temperature before opening. The dried pellet is resolubilized in 3 µl DMF or DMSO, briefly vortexed and added to the amino-modified cDNA in coupling buffer. The mixture is again vortexted and incubated in the dark for 1 hour at room temperature. Although the tetrafluorphenole activated dyes are more stable in aqueous solution as compared to the NHS esters, the resolubilized dye should **NOT** be further aliquoted or stored for next day experiments.

Purification of the dye-labeled cDNA.

The fluorescent dye labeled cDNA can be purified as the amino-modified cDNA using column purification or ethanol precipitation.

Determination of the label degree

The relative efficiency of the coupling reaction may be assessed by measuring the absorbance of the nucleic acids at λ=260 nm and the absorbance of the dye at it's absorbance maximum (λ_{max}). According to the Lambert-Beer law A = C x path length (usually 1 cm) x ε, with C = concentration (M) and ε = extinction coefficient (cm^{-1} x M^{-1}) the concentration of the nucleic acids and the coupled fluorophor can be calculated.

The concentration of the conjugate can be determined by measuring the absorbance at A_{260}. The absorbance of the nucleic acids (average) has to be corrected by the absorbance of the dye at λ=260 nm, which is expressed as correction factor $C_{f\ 260}$ (A_{260} free dye/A_{max} free dye).

$$C_{base} = \frac{A_{260} - (C_{f\ 260} \times A_{max})}{\varepsilon_{base}}$$

The ε_{base} is 6,600 (cm^{-1}*M^{-1}) for dsDNA and 8,500 (cm^{-1}*M^{-1}) for ssRNA.

$$C_{dye} = \frac{A_{max}}{\varepsilon_{dye}}$$

The ε_{dye} values for the Oyster®-550 and Oyster®-650 TFP are listed below. With this the label degree (ratio dye:base) can be calculated:

$$\text{label degree (D/N)} = \frac{C_{dye}}{C_{base}}$$

The label degree for hybridization experiments on microarrays is ideally between 0.05 and 0.1.

Spectral characteristics of the Oyster®-dyes used in array kits

Dye	ε_{dye} ($cm^{-1} \times M^{-1}$)	A_{max}* (nm)	E_m* (nm)	$C_{f\,260}$	spectrally similar dyes
Oyster®-550	150 000	553	572	0.05	Cy3™, TRITC, TAMRA, Alexa Fluor® 555
Oyster®-650	200 000	653	672	0.04	Cy5™, Alexa Fluor® 647

* nucleotide bound dye

ε, extinction coefficient; A_{max}, absorbance maximum; E_m, emission maximum; $C_{f\,260}$, correction factor at 260 nm, A_{260} of the free dye/A_{max} of the free dye.

Cy3™ and Cy5™ are trademarks of GE Healthcare, Alexa Fluor® is a registered trademark of Invitrogen Inc.

Several of Denovo Biolabels products and product applications are covered by German and foreign patents and patents pending. No licensing agreement is necessary for use of Oyster®-500, -556, -645 and -656 as long as our products are utilized to produce another value added product for research or resale. For resale of our unmodified products as well as for all products involving Oyster®-550 or -650, a specific agreement or licensing agreement from Denovo Biolabels is necessary. All names containing the designation ® are registered with the German Patent and Trademark Office.

Copyright 2005, Denovo Biolabels GmbH. All rights reserved. This information is subject to change without notice.

For further information please contact our technical support

Denovo Biolabels GmbH
Mendelstr. 7
48149 Muenster, Germany

Phone +49 (0)251 980 2918
Fax +49 (0)251 980 2917

or visit our website: **www.biolabels.com** info@biolabels.com