

Labeling of oligonucleotides

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The Oyster[®] fluorophores come ready to use as mono- or bifunctional NHS-esters in a sealed alumina bag containing desiccant and should be stored at 4°C. One vial (extension 1xV of the order number) contains 0.2 mg activated fluorophor, which is sufficient to label up to 40 nmol aminoallyl modified oligonucleotides. The excess of dye has to be optimized individually. We recommend to start with a 10 fold molar excess of the dyes of the aminomodified compound. Please note, that the Oyster 645 is bifunctional and thus may be downscaled accordingly.

Preparation of oligonucleotides

In most cases the oligonucleotides are purchased from commercial suppliers. For the reaction with the activated Oyster[®] fluorophores, make sure that the oligonucleotides are modified with amino groups at the 3' or 5' end. The yield of 5' end amino modified conjugates is reported to be higher compared to 3' modified oligos.

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The dye is covalently coupled to the primary amino residues of the modified oligonucleotides.

The preactivated dye is moisture sensitive. Upon opening of the eppendorf cup, add 20 µl of dimethylformamide (DMF) or dimethylsulfoxide (DMSO) to resolubilize the dye. The modified oligonucleotide (5 - 40 nmol), dissolved in 200 µl 0.1 M sodium bicarbonate buffer, pH 8.0, is added directly to the vial.

The new Oyster-550 DNA and Oyster-650 DNA are significantly more hydrophilic and might be dissolved in distilled water for further aliquotation (use dye solution within 10 minutes!) or used directly with the oligonucleotide solved in bicarbonate buffer.

Warning:

Aqueous solutions are **not** suitable for storage of activated dyes. Under alkaline conditions, the activation level decreases rapidly, e.g. incubation at pH 10.0 lead to up to 90 % hydrolysis within 10 min.

Note: Make sure that no other reagents with primary amine groups are present in the reaction mix, as they will compete with the amino-modified oligonucleotide. Check pH!

The vial is shaken continuously or from time to time at ambient temperature. After 2 h incubation the complete mixture is purified as described

below. A quenching of remaining activated dye with ethanolamine is not necessary.

Purification of labeled oligonucleotide

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 nucleotides may be purified by repetitive ethanol precipitation.

Twenty µl of 3 M NaCl and 250 µl chilled ethanol are added to the reaction mixture. The solution is mixed and cooled for at least 30 min. at -20°C. Upon centrifugation for 30 min at 14.000 g, the supernatant is separated and discarded. The pellet is washed twice with cold ethanol (70 %), dried (be sure that the pellet is not completely dry, otherwise resolubilization may be difficult) and resolubilized in 200 µl Tris/HCl buffer, pH 8.0.

C) If available, the purification may be carried out by HPLC-separation on a reversed phase column (C-18) using an acetonitrile/H₂O (80:20, v/v) gradient.

Determination of the label degree

The relative efficiency of the coupling reaction may be assessed by measuring the absorbance of the oligonucleotide at λ=260 nm and the absorbance of the dye at its absorbance maximum (λ_{max}). According to the Lambert-Beer law $A = C \times \text{path length (usually 1 cm)} \times \epsilon$, with C = concentration (M) and ε = extinction coefficient (cm⁻¹ x M⁻¹) the concentration of the oligonucleotide and the coupled fluorophor can be calculated.

The concentration of the conjugate can be determined by measuring the absorbance at A₂₆₀. The absorbance of the oligonucleotide has to be corrected by the absorbance of the dye at λ=260 nm, which is expressed as correction factor C_{f 260} (A₂₆₀ free dye/A_{max} free dye). The correction factors for the Oyster[®] fluorophors are listed below.

$$C_{\text{nuc}} = \frac{A_{260} - (C_{f\ 260} \times A_{\text{max}})}{\epsilon_{\text{nuc}}}$$

(The ε_{nuc} will vary for different oligonucleotides and is usually provided by the supplier of the oligonucleotides. If you want to calculate the ε_{nuc} according to the Lambert-Beer law on your own,

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you may find useful tools under, e.g. <http://www.pitt.edu/~rsup/OligoCalc.html> or <http://www.basic.nwu.edu/biotools/oligocalc.html>)

$$C_{\text{dye}} = \frac{A_{\text{max}}}{\epsilon_{\text{dye}}}$$

With this the label degree (moles dye per moles nucleotide, D/N) can be calculated:

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

The postsynthetic coupling of activated fluorescent dyes to aminoallyl modified oligonucleotides typically yield a label degree of 0.6.

Spectral characteristics of the Oyster[®]-dyes:

	ϵ ($\text{cm}^{-1} \times \text{M}^{-1}$)	A_{max}^* (nm)	E_{m}^* (nm)	$C_{\text{f } 260}$
Oyster [®] -500	78,000	503	528	0.29
Oyster [®] -550	150,000	553	570	0.05
Oyster [®] -556	155,000	560	570	0.03
Oyster [®] -650	200,000	653	670	0.04
Oyster [®] -645	220,000	649	666	0.05
Oyster [®] -656	200,000	660	675	0.03

* nucleotide bound dye

Reagents

0.1 M sodium bicarbonate buffer, pH 8.0

dry dimethylformamide

10 mM Tris/HCl buffer, pH 8.0

absolute Ethanol

70% (v/v) Ethanol

3M NaCl

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.

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Application note 2



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