Application note 1

Labelling of antibodies

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In contrast to other commercially available fluorophores our new developed Oyster® labels are characterized by a reduced tendency to form aggregates, thus preventing internal quenching phenomena. Consequently, high label degrees (D/P ≥ 6) may be realized without loss of brightness.

The dye comes ready to use as mono- or bifunctional NHS-esters in a sealed alumina bag containing desiccant and should be stored at 4°C. The amount of fluorophor (~ 0.2 mg) is sufficient to label at least 1.5 mg of antibody.

Preparation of antibodies

The antibody is dialyzed into 10 mM phosphate buffer, pH 7.4 to 8, depending on your desired label degree. For higher pH, borate buffer may be used as well.

The fluorophor is covalently coupled to primary amines (lysines) of the immunoglobuline.

Note: Small amounts of sodium azide may be tolerated due to the large molar excess of the dye over the protein. Keep in mind, however, that all buffers (TRIS) and additives carrying additional amines and other nucleophiles may prevent the reaction with your target protein.

The concentration of the antibody should be 1 mg/ml or higher. (For IgG, 1 mg/ml has an A_{280} of 1.4)

Conjugation of antibodies

The pre-activated dye is moisture sensitive. Upon opening of the eppendorf cup, add 20 µl of dimethylformamide (DMF) or dimethylsulfoxide (DMSO) to resolubilize the fluorophore. The solubilized dye is added directly to the dialyzed antibody solution at the desired pH (pH 8.0 is recommended to start with).

The new Oyster-550 NHS and Oyster-650 NHS are significantly more hydrophilic as compared to the other Oyster dyes and might be dissolved in distilled water or buffer prior to use. In order to enable reproducible coupling results make sure that the dye is completely dissolved before adding the dye to the protein solution.

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: If your antibody is available at a high concentration, you may dilute the antibody with the coupling buffer. Check pH!

Short protocol
The vial is shaken continuously or from time to time at ambient temperature. After 1 h incubation, activated NHS-esters are blocked by addition of 20 µl 10 % glycine solution (w/w). The complete mixture is purified as described below.

Extended protocol
For some applications, e.g. the development of fluorescence immuno assays, the best compromise between remaining affinity of a given conjugate and a high label degree has to be elucidated. In this case we recommend to follow a time course, where , e.g, 4 aliquots are prepared by taking samples after 10, 20, 40 and 60 min. The reaction of each aliquot is quenched with 10 % glycine solution. This procedure allows for a separate purification and testing of conjugates. A second batch may be prepared afterwards under optimized conditions.

Purification of conjugates

For the purification of conjugates, we recommend to use size exclusion chromatography, e.g., G-25™ or Nap 10™ or a ready to use dialysis chamber (Slide-A-Lyzer™).

Determination of the label degree

The relative efficiency of your coupling reaction may be assessed by measuring the absorbance of the protein at λ=280 nm and the absorbance of the dye at its 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 your protein and the bound fluorophor can be calculated.

Dilute a small sample of your conjugate to a concentration of about 0.1 to 0.2 mg/ml. The concentration of your protein conjugate can be determined by measuring the absorbance at A_{280}. The absorbance of the protein has to be corrected by the absorbance of the fluorophor at λ=280 nm, which is expressed as correction factor $C_{f\ 280}$ (A_{280} free fluorophor/A_{max} free fluorophor). The correction factors for the Oyster® dyes are listed below.

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$$C_{prot} = \frac{A_{280} - (C_{f\ 280} \times A_{max})}{\varepsilon_{prot}}$$

(with ε = 210 000 cm^{-1} x M^{-1} for a typical IgG (which corresponds to A_{280} of 1.4 for a 1 mg/ml solution) and a 1 cm cuvette. The extinction coefficient will vary for different proteins.

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

With this the label degree (moles fluorophor or dye per mol protein, D/P) can be calculated:

$$\text{label degree (D/P)} = \frac{C_{fluorophor}}{C_{protein}}$$

Spectral characteristics of the Oyster®-dyes

	ε (cm^{-1} x M^{-1})	A_{max}* (nm)	Em* (nm)	$C_{f\ 280}$
Oyster®-500	78,000	505	530	0.21
Oyster®-550	150,000	555	574	0.06
Oyster®-556	155,000	562	575	0.04
Oyster®-650	200,000	655	574	0.06
Oyster®-645	220,000	650	669	0.06
Oyster®-656	200,000	662	679	0.04

* protein bound dye

Best results are obtained with label degrees between 6 and 10.

Reagents

10 mM phosphate buffer, pH 7.4 to 8

dimethylformamide or
dimethylsulfoxide
(free of amine contamination!!)

glycine

For further information please contact our technical support

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