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Product Information

75824 Tracy 652 Protein Labeling Kit

Introduction

The Tracy 652 Protein Labeling Kit provides a fast labeling of purified proteins as well as an easy purification of the resulting conjugate. The succinimidyl ester group of the dye enables an effective reaction with primary amino groups of the protein. The kit contains everything for 5 labelings including purification procedures. It is optimized for 1 mg protein per reaction. A final molar dye / protein (D/P) ratio between 2 – 9 can be expected, depending on the kind of protein [1)].

Kit components

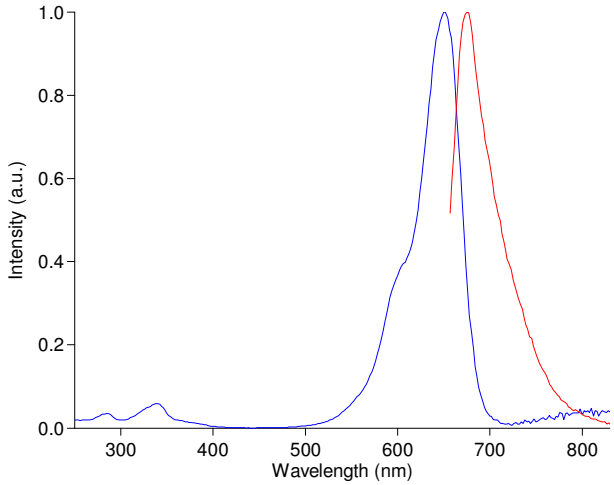
A) 5 vials Tracy 652 reactive dye, each containing 0.24 mg dye (optimized for 1 mg protein), product no. 42454
B) Sodium bicarbonate buffer solution, pH 9.5, 10 ml, product no. 88975
C) Phosphate buffer solution, pH 7.5, 100 ml, product no. 76847
D) 5 gel filtration columns (PD-10), product no. 73226
E) Application note

Storage

Store the kit at 4 °C, protected from moisture. Protect the reactive dye from light.

Label characteristics

Tracy 652 NHS ester (product no. 42454)
$\lambda_{abs\ max}$ = 648 nm (in 0.1 M phosphate buffer solution pH 7.0)
$\lambda_{em\ max}$ = 670 nm (in 0.1 M phosphate buffer solution pH 7.0)
ε = 181 000 $cm^{-1}M^{-1}$



Labeling protocol

1. Dissolve 2 – 10 mg/ml protein in sodium bicarbonate buffer solution (B). Protein concentration < 2 mg/ml will decrease the labeling efficiency of the labeling reaction. The protein solution has to be free of amino group-containing substances (e.g. Tris buffer solution or ammonium ions). Azide with concentration < 3 mM will not affect the labeling efficiency.
2. Dissolve the reactive dye (A) in 20 µl sodium bicarbonate buffer (B). Proceed quickly with the labeling procedure, so that there is no chance for hydrolysis before coupling to the protein.
3. Transfer the protein solution to the vial containing the reactive dye.
4. Incubate the reaction mixture for 2 hours while gently stirring and protecting the vial from light. It is recommended to prepare the dye solution immediately before starting the coupling reaction. Due to the different reactivity of proteins, the degree of labeling might vary between 2 – 9. The incubation time can be prolonged up to 12 h at 4 °C.

Separation of protein-dye-conjugates from free dye

The gel filtration columns are used in order to achieve good separation of the protein-dye-conjugates from excess free dye (size exclusion limit: 5 kDa). Solvent flow in the columns is achieved by gravity only, there's no need to apply pressure.

1. Remove the top cap and pour off excess liquid (contains 0.15 % Kathon$^{TM\ 2)}$; take care for safety issues, wear gloves)
2. Remove the bottom cap, then cut off the bottom tip of the column (D)
3. Equilibrate the column first with 10 ml water, then with 15 ml phosphate buffer (C). Discard the flow-through.
4. Add the labeling mixture to the column and allow to infiltrate
5. Add 5ml phosphate buffer (C) and start the elution
6. Separation between a first band (dye-labeled protein) and a second band (free dye) will become visible
7. Collect the dye-labeled protein fraction (= first band)

Determination of dye/protein (D/P) ratio

After a successful separation of the free dye from the labeled protein, the dye to protein ratio (D/P) of the conjugate can be calculated:
Measure the absorbance of the conjugates at 280 nm and 648 nm. Before measuring, the conjugate should be diluted so that the maximum absorbance measured is between 0.5 and 1.0 A.U.

The D/P molar ratio can be calculated as follows:

$$D/P = \frac{A_{648} \times \varepsilon_{Protein}}{\left[A_{280} - (A_{648} \times 0.044)\right] \times 181{,}000}$$

A_{648} = absorbance at 648 nm measured in a cuvette with a pathlength of 1 cm
A_{280} = absorbance at 280 nm measured in a cuvette with a pathlength of 1 cm
$\varepsilon_{Protein}$ = molar extinction coefficient of the protein at 280 nm [$cm^{-1}M^{-1}$]. IgG usually have ε of 203,000.
181,000 = molar extinction coefficient (ε_{dye}) of the Tracy 652 dye at 648 nm [$cm^{-1}M^{-1}$]
0.044 = correction factor due to the fluorophore's absorbance at 280 nm

The final protein concentration can be calculated as follows:

$$c_{\mathrm{Pr}otein}\ (mg/ml) = \frac{A_{280} - (0.044 \times A_{648})}{\varepsilon_{\mathrm{Pr}otein}} \times MW_{\mathrm{Pr}otein} \times Dilution\ \ factor$$

MW_{Protein} = Molecular weight of the protein (for IgG 150,000 g / mol)
Dilution factor = Dilution of the labeled conjugate prior to measurement by spectrophotometer

Handling of labeled conjugates

Labeled conjugates should be stored at 4 °C and protected from light. To improve stability, sodium azide (0.01 %) may be added. Depending on the type of protein, the conjugates can be stable for several months. For extended storage, the solution should be kept at –20 °C. Avoid repeated freeze-thaw cycles. In order to prevent denaturation of the conjugates add bovine serum albumin (BSA) to a final concentration of 1 – 10 mg / ml or use one of our BioStab stabilizers.

References

1) E. Harlow, D. Lane, Using *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, **1999**
2) Kathon is a registered trademark of Rohm and Haas Co.

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.