Cyanine 3-NHS Ester Pack

Reagents for indirect labeling of probes with cyanine 3-NHS ester via post labeling method

Cat. No. 42541

12 x 50 nmoles dried dye

For Research Use Only

INTRODUCTION

Cyanine dyes, by virtue of their strong fluorescence and water solubility, are excellent fluorescent labels for biological assays. The dyes are extremely useful for labeling nucleic acids, nucleotides and proteins. Highly efficient nucleic acid labeling can be achieved by using an NHS ester of a cyanine dye to label nucleic acids that have been modified by the incorporation of reactive allylamine groups. This procedure has found wide use in labeling for array analysis.

The Cyanine 3-NHS Ester Pack contains 12 vials of packaged highly reactive cyanine 3-NHS ester in dry form that is easily prepared for labeling reactive amino groups.

COMPONENTS

Twelve foil packets: Each containing 50 nmoles of dried cyanine 3-NHS ester.

Product specification sheet: with recommendations for using the cyanine 3-NHS ester

STORAGE

Store the reagents at 2-8°C in the dark. Do not use if desiccant in foil pack is pink.

SAFETY WARNINGS AND PRECAUTIONS

Warning: The reagents provided herein are for research purposes only and are not recommended or intended for diagnosis of human and animal disease. The reagents provided are not to be administered internally or externally in humans or animals.

Treat all chemicals as potentially hazardous. Only users trained in laboratory techniques and who follow good laboratory practices should handle the product. Protective clothing, *e.g.*, overalls, safety glasses and gloves, should be worn. Be careful to avoid contact with skin or eyes. However, if contact occurs be sure to wash skin or eyes immediately and thoroughly with water.

Take care in handling reagents to prevent staining of skin, clothing and other items.

Caution: Because the NHS ester dye is intensely colored and extremely reactive, it can be easily degraded by air and humidity after the vial is opened and re-capped. We therefore recommend discarding any unused dissolved dye.

RECOMMENDED CONDITIONS FOR COUPLING CYANINE 3-NHS ESTER TO TARGET MOLECULES

1. pH

   Coupling reactions are optimal at pH 9.0 to 9.5. Carbonate buffer systems are sufficient to achieve this. If necessary, coupling can be performed at a pH as low as 7.3 but will take longer to reach completion.

2. Volume of coupling reagent

   The coupling reaction should be performed in a small volume in order to maintain a high concentration of reactants. To achieve this, the sample can be dried and dissolved in 10 µL of coupling buffer. Add 3 µL of the cyanine 3-NHS ester in DMSO.

3. DMSO concentration

   Lyophilized cyanine dyes should be dissolved in a minimal volume of DMSO. A concentration of less than 25% DMSO is optimal.

4. Prevent bubble formation

   Cyanine dye esters can be inactivated by oxygen in the atmosphere. To avoid this, mix or vortex gently to prevent excessive bubble formation.

5. Protect from light

   Perform coupling reactions in a closed drawer or box to prevent exposure to light. After clean-up procedures, minimize exposure to ambient light.

6. Primary amino-containing contaminants (Tris, glycine, or any free amino acids)

   The cyanine dye esters react with primary amino groups present on target proteins or on allylamine-labeled nucleotide polymers. Buffers containing primary amine groups will compete in the conjugation reaction. Care should be taken to eliminate contaminating reactive groups.

CONJUGATION OF DYE TO ALLYLAMINE-CONTAINING NUCLEIC ACIDS

Perform all steps at room temperature. The allylamine-containing nucleic acid sample should be in a small volume of coupling buffer (10 µL or less) at pH 9-9.5. Dissolve the dye NHS ester in 3 µL of DMSO, and add to the allylamine-containing sample and mix thoroughly. Incubate in the dark for 1 hour. Mix every 15 minutes. Following incubation, increase the volume and adjust the pH as required for the purification method to be used.

CONSIDERATIONS FOR VARIOUS PURIFICATION METHODS

Following the coupling reaction, the reaction volume should be increased to 70-80 µL with water prior to purification. A longer column is more efficient for the separation of free cyanine dyes from labeled product. We recommend Sephadex G-25 or BioGel P-4 that contain the appropriate exclusion size for trapping free nucleotides.

Purification columns such as Qiagen GFX, to which the sample is bound and then eluted, require a sample pH that is lower than the optimum pH of the coupling reaction. The addition of 3M sodium acetate, pH 5.3, can be used to adjust the pH of the reaction mix to 5.3, appropriate for efficient sample binding to the column.

CALCULATIONS

After purification, the amount of coupled cyanine 3 dye in pmoles/µL can be calculated by the following formula:

$$\text{pmoles Cyanine 3 per µL} = \frac{\text{Abs}_{550} \text{ x dilution factor}}{0.145}$$

To calculate the specific fluorescence activity, first determine the concentration of protein, DNA or RNA and then calculate the ratio of dye to target molecule.

FLUORESCENCE QUENCHING

Conjugated cyanine dye molecules in close proximity undergo energy transfer resulting in a sharp decrease in signal strength. Signal strength can be maximized by determining the optimum ratio of allylamine-labeled nucleotide to its cognate nucleotide in the incorporation reaction. This can be done in a series of reactions in which the ratio of allylamine-labeled nucleotide to its cognate nucleotide is varied, and a constant amount dye NHS ester is used for conjugation to each sample. After purification and quantification of the labeled DNA, the picomoles of dye incorporated for each of the samples can be determined by absorption measurements. The fluorescence of each sample, where all samples contain the same amount of conjugated dye, is then determined.

Technical Support

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Notice to Purchaser

Limited Warranty

Patents

This product or use of this product is covered by one or more claims of Enzo patents including, but not limited to the following: U.S. Patent Nos. 4,994,373 and 5,175,269, and patents pending.

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