GE Healthcare

# Amersham Cy3 Mono-Reactive Dye Protein Array Grade

## Reagents for the labelling of proteins for protein arrays with Cy™3 monofunctional dye

## Product Specification Sheet

Code: 25-8009-86

**Warning**
**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.**

## Storage

Store refrigerated at 2–8°C in the dark.

## Expiry

See outer packaging.

## Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Caution: This dye is intensely coloured and very reactive. Care should be exercised when handling the dye vial to avoid staining clothing, skin and other items.

## Components

- Cy3 mono-reactive dye; 2 securitainers each containing dried dye to label 1 mg of protein
- Instructions for use

## Other materials required

- Conjugation buffer: 0.1 M Sodium Carbonate buffer (pH 9.3)
- Separation column containing a permeation gel (Sephadex™ G-50, or Bio-Gel™ P-10, minimum of 1 cm diameter and 12 cm length packed volume)
- Separation buffer: Phosphate-Buffered Saline, pH 7.2, containing 0.1% Sodium Azide
- Test tubes, transfer pipettes, glassware

## Introduction

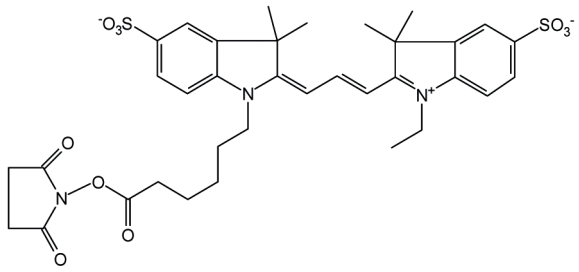
Protein arrays offer a solution to accurate protein profiling and analysis of complex samples, complementing existing technologies, such as two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) and DNA microarrays.

Protein arrays are already used in disease profiling, where differential expression of specific proteins is measured and correlated to a particular disease state or drug treatment. They can also be used in many stages of the drug discovery process, including target identification and validation, toxicology, pathway elucidation, mode of action, and to look at drug resistance and surrogate markers of drug response. CyDye reagents are important as fluorescent labels for biological compounds (1, 4). CyDye fluors are intensely fluorescent and highly water soluble, providing significant advantages over other existing fluorophores (3). CyDye fluors form the core of many key technologies and platforms, in fields as diverse as DNA microarrays, 2-D DIGE, DNA sequencing, high-throughput screening, flow cytometry, and now protein arrays, where they are particularly suited to highly sensitive and accurate differential analysis.

The Cy3 dye is an orange fluorescing Cyanine that produces an intense signal easily detected using most Rhodamine filter sets.

The Cy3 dye supplied here is a monofunctional NHS-Ester, and is provided in a dried, pre-measured form ready for the labelling of compounds containing free amino groups.

**Figure 1** Cy3 monofunctional dye




## Recommended procedure for use

This protocol has been designed for the preparation of Cy3-labelled proteins, and is ideally suited for protein arrays. It is designed to label 1 mg protein to a final molar dye/protein (D/P) ratio between 4 and 12. This assumes an average protein molecular weight of 155 000 Daltons. Other D/P ratios can be obtained by using different amounts of protein.

**NOTE:** The following materials and procedures have been optimized for IgG antibodies. Other proteins may also be readily labelled (5, 6). However, choice of buffers, separation media, and technique may vary in order to produce optimal results.

Altering the protein concentration and reaction pH will change the labelling efficiency of the reaction. Optimal labelling generally occurs at pH 9.3. Proteins have been successfully labelled with this dye at a pH as low as 7.3, however, labelling times must be significantly longer at lower pH (1). Higher protein concentrations usually increase labelling efficiency. Solutions of up to 10 mg/ml protein have produced good conjugation reactions.

## Conjugation of dye to protein

Protein to be conjugated should be dissolved at 1 mg/ml in Sodium Carbonate-Sodium Bicarbonate buffer (2). Add the protein solution (1 ml) to the dye vial, cap the vial, and mix thoroughly. Care should be taken to prevent foaming of the protein solution. Incubate the reaction at room temperature for 30 minutes with additional mixing approximately every 10 minutes.

**NOTE:** Buffers containing primary amino groups such as TRIS and glycine will inhibit the conjugation reaction. The presence of low concentrations (<2%) of biocides such as Azide or Thimerosal do not affect protein labelling.

## Separation of protein from free dye

Labelled protein can be separated from the excess, unconjugated dye by gel filtration. It is convenient to pre-equilibrate the column (eg Sephadex G-50 - see "Other materials required") with Phosphate-Buffered Saline and to elute the protein using the same buffer. Two pink bands should develop during elution. The faster moving band is Cy3-labelled protein, whilst the slower band is free dye. Many Cy3-labelled proteins can be stored at 2–8°C without further manipulation.

Spin-column chromatography (Microspin G-25 columns) can be used to remove excess dye, but as with any sample manipulation post-labelling, could also lead to loss or misrepresentation of proteins.

Labelled protein can also be separated from unconjugated dye by dialysis. Dialysis does not give as efficient and rapid a separation as gel filtration. We therefore recommend that protein purification by gel filtration be used.

## Estimation of final dye/protein (D/P) ratio

Dilute a portion of the labelled protein solution so that the maximum absorbance is 0.5 to 1.5 AU. Molar concentrations of dye and protein are calculated, and the ratio of these values is the average number of dye molecules coupled to each protein molecule. Molar extinction coefficients of 150 000 $M^{-1}$ $cm^{-1}$ at 552 nm for the Cy3 dye and 170 000 $M^{-1}$ $cm^{-1}$ at 280 nm for the protein are used in this example. The extinction coefficient will vary for different proteins. The calculation is corrected for the absorbance of the dye at 280 nm (approximately 8% of the absorbance at 552 nm).

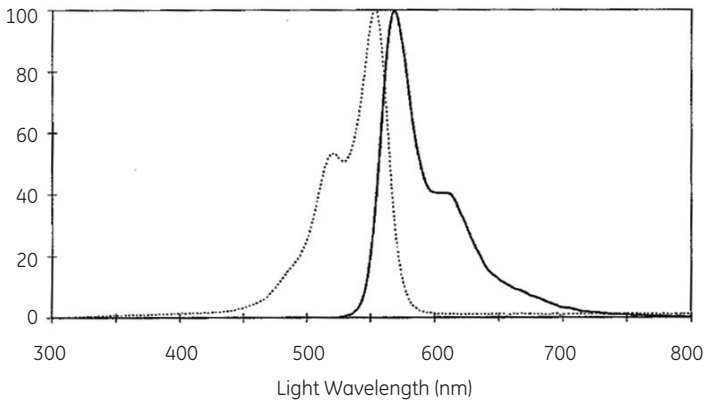
$[\text{Cy3 dye}] = (A_{552}) / 150\,000$

$[\text{antibody} = (A_{280}\text{-}(0.08 \bullet (A_{552})] / 170\,000$

$(D/P)_{final} = [\text{dye}] / [\text{antibody}]$

$(D/P)_{final} = (1.13 \bullet (A_{552})\ ] / [A_{280}\text{-}(0.08 \bullet (A_{552})]$

**Figure 2** Cy3 dye absorption and fluorescence spectra




**Cy3 monofunctional dye characteristics**

| | |
|---|---|
| Formula weight | 765.95 |
| Absorbance max | 550 nm |
| Extinction max | 150 000 $M^{-1}cm^{-1}$ |
| Emission max | 570 nm |
| Quantum yield | >0.15* |

* for labelled proteins, D/P = 2

## References


1. Mujumdar, R.B. *et al., Cyanine dye labelling reagents: Sulfoindocyanine Succinimidyl Esters Bioconjugate Chemistry* **4** (2), 105-111 (1993).
2. Southwick, P.L. *et al., Cyanine dye labelling reagents: Carboxymethylindocyanine Succinimidyl Esters Cytometry* **11**, 418-430 (1990).
3. Wessendorf, M.W. and Brelje, T.C., *Which fluorophore is brightest? A comparison of the staining obtained using Fluorescein, Tetramethylrhodamine, Lissamine Rhodamine, Texas red, and Cyanine 3.18 Histochemistry* **98** (2), 81-85 (1992).
4. Yu, H. *et al., Cyanine dye dUTP analogs for enzymatic labelling of DNA probes Nucleic Acids Research* **22** (15), 3226-3232 (1994).
5. Haab, B.B. *et al., Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions Genome Biology* **2** (2), research 0004.1-0004.13 (2001).
6. MacBeath, G. and Schreiber, S.L., *Printing proteins as microarrays for high-throughput function determination Science* **289**, 1760-1763 (2000).


## Related products

| | |
|---|---|
| Cy5 Mono-Reactive Dye Protein Array Grade | 25-8009-87 |
| Microspin G-25 Columns | 27-5325-01 |

## Legal

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