# **Cell Separation and Cellular Analysis**

# Fluorescent dyes and labelling

## Fluorescein dyes and labelling kits, Thermo Scientific Pierce

# Thermo

Fluorescein dyes are used in wide ranging applications including fluorescence microscopy, flow cytometry and immunofluorescence based assays such as Western blotting and

FITC and NHS-fluorescein are reactive toward primary amine groups on proteins, peptides and other biomolecules, while fluorescein-5-maleimide and 5-IAF react with free sulfhydryls on cysteine residues.

Most activated fluorescein derivatives are mixture of isomers with reactive groups attached at the 5- and 6-positions of the bottom ring. The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer. The single isomer is used in high resolution separation techniques such as sequencing and capillary electrophoresis.

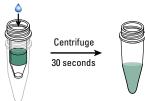


Step 1. Labelling reaction



Add antibody to vial containing pre-measured dye. Incubate 1 hour at room temperature.

## Step 2. Removal of excess fluorescent dye



Apply labelling reaction to Spin Desalting Column.

labelled antibody

## Protocol summary for Thermo Scientific Pierce fluorescent antibody

### Properties of activated fluorescein dyes

Fluorescein derivative	MW	Ex/Em maxima, nm
FITC (Fluorescein Isothiocyanate)	389.2	494/518
NHS-Fluorescein	473.4	494/518
5-lodoacetamido-fluorescein (5-IAF)	515.3	494/518
Fluorescein-5-maleimide	515.3	494/518

Catalogue No	Description
PN46424	FITC, 1g
PN46425	FITC, 100mg
PN46409	NHS-Fluorescein, 1g
PN46410	NHS-Fluorescein, 100mg
PN62246	5-lodoacetamido-fluorescein (5-IAF), 25mg
PN62245	Fluorescein-5-maleimide, 25mg
PN53027	FITC antibody labelling kit Includes: FITC, 3 vials Borate buffer, 1mL Spin columns, x 6 Microcentrifuge collection tubes, x 12 Purification resin, 5mL
PN53029	Fluorescein antibody labelling kit Includes: NHS Fluorescein, 3 vials Borate buffer, 1mL Spin columns, x 6 Microcentrifuge collection tubes, x 12 Purification resin, 5mL

## Rhodamine dyes and labelling kits, Thermo Scientific Pierce

## Thermo SCIENTIFIC





TRITC, NHS-rhodamine and Texas Red™ Sulfonyl Chloride are reactive towards primary amine groups on proteins, peptides and other biomolecules.

Rhodamine dyes are often a mixture of isomers with reactive groups attached at the 5- and 6-positions of the bottom ring. The properties of these isomers are indistinguishable in terms of excitation and emission spectra and for protein applications there is no need to isolate a specific isomer. The single isomer is used in high resolution separation techniques such as sequencing and capillary electrophoresis.

## Properties of activated rhodamine dyes

Rhodamine derivative	MW	Ex/Em maxima, nm
TRITC (Tetramethylrhodamine Isothiocyanate)	443.5 (Supplied as chloride salt, MW 479.0)	544/572
NHS-Rhodamine	527.5	552/575
Texas Red Sulfonyl Chloride	625.2	596/615

Catalogue No	Description	Quantity
PN46112	TRITC	10mg
PN46406	NHS-Rhodamine	25mg
PN46115	Texas Red Sulfonyl Chloride	10mg
PN53031	Rhodamine antibody labelling kit Efficiently label and purify 3 x 1mg of IgG or other protein in 1hr. Includes: NHS Rhodamine, 3 vials Borate buffer, 1mL Spin columns, x6 Microcentrifuge collection tubes, x12 Purification resin, 5mL	Kit

