# IRIS™ 3.5-NHS active ester

Product code: 35WS-02

## Product specification sheet

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.

## Product description

**IRIS™ Dyes Active Esters** are succinimidyl derivatives of IRIS™ Dyes. They are suitable for conjugation of any biomolecules carrying free primary amines, such as proteins, peptides, amino-modified antibodies, biopolymers.
The dyes have absorption and emission maxima in the visible and near infrared region of the spectrum. Due to the wide range of dyes offered by Molecular Targeting Technologies, with different chemical-physical properties, it is possible to find the right product for any biological application involving fluorescence analysis. IRIS™ active esters are intensely coloured and stain on contact: always wear gloves when working; due to their water-solubility, clean-up with water and detergents is suggested.

## Storage and handling

1. IRIS™ active esters hydrolyse in aqueous environment: store the dyes as much dry as possible.
2. The dyes should be dissolved in anhydrous solvents, such as DMF (N,N'-dimethyl formamide) or DMSO (dimethyl sulfoxide), in which they are stable for few days if stored at +4°C or –20°C. In some applications (e.g. for the conjugation of particular proteins insoluble in DMF) the dyes can be dissolved in aqueous buffers and used immediately. In water the dyes hydrolyses within one hour.
3. Avoid direct exposition to ambient or artificial light and pH above 9.

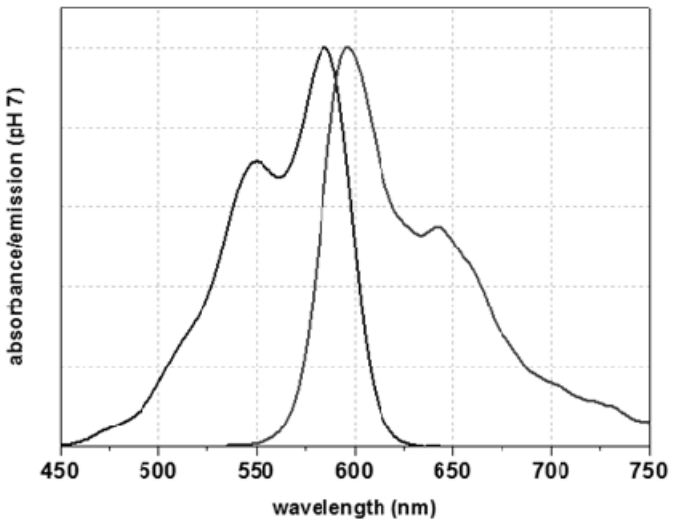


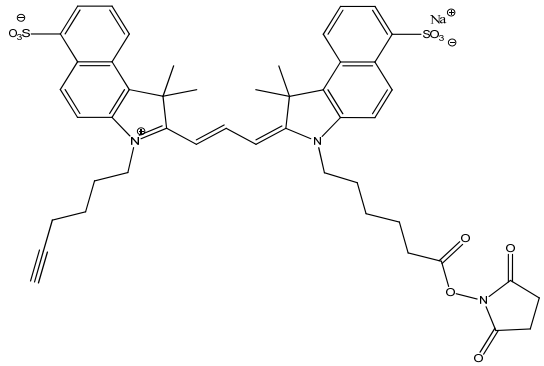

Fig.1 Excitation and Emission spectra

## Components

Each package includes one microcentrifuge tube of IRIS™-NHS active ester provided in a liophylized format. The amount in weight could be 1 mg or 5 mg, depending on the ordered product.

### IRIS™ 3.5-NHS active ester characteristics

| | |
|---|---|
| Formula weight: | 902.02 g/mol |
| Absorbance max: | 578 nm |
| Extinction max: | 150,000 $M^{-1}cm^{-1}$ |
| Emission max: | 592 nm |
| Quantum yield: | >0.15 |



## Preparation and use

### Conjugation of proteins

Here is described a general protocol for conjugation of proteins as antibodies, receptors, ligands, carrier proteins. Conditions for any specific case should be adapted by the operators, especially regard to the F/P ratio desired. It indicates the number of fluorochrome molecules per molecule of protein. This value should be measured for all derivatives prepared with fluorescent tags. The F/P ratio is an important factor in predicting the behaviour of fluorochrome labelled proteins.
For antibodies, e.g., optimal F/P value is about 1.

- Dissolve the protein to be labelled at a concentration of 1 mg/ml in 0.1 M Sodium Borate pH 8.5. If your protein is stored in sodium azide or amine-containing buffers (e.g. Tris buffer), dialyze or desalt your sample. Dissolve the active ester in dry DMF at a concentration of 10 mg/ml. Add the dye to the protein in amount suitable to have a 10-fold molar excess of dye. This excess should ensure a F/P ratio of about 1. If a higher F/P value is desired, you can use a higher excess of dye.
- Wrap the reaction tube with aluminium foil to avoid contact with light.
- React and rotate for 2 hours at room temperature
- Purify the labelled protein from non reacted dye by gel-permeation on a Sephadex™ G-25 column. Labelled protein is the first eluting band.

### Conjugation of amino-modified Oligonucleotides

At basic pH conjugation occurs virtually exclusively at the amino group and not at all the exocyclic amino groups of the nucleosides.
Dissolve the product from a 200 nmoles (or similar) synthesis of amino-modified oligonucleotides (i.e. 100-200 nmoles of free primary amines) in 800 ml of 0.1 M Sodium Borate pH 8.5.
Freshly prepare a 10 mg/ml solution of active ester in dry DMF. Add 200 ml of the solution to the reaction misture.
Wrap the reaction tube with aluminium foil to avoid contact with light.
React and rotate for at least 2 hours (up to overnight) at room temperature.
Purify the labelled oligo according to standard desalting methods: on Sephadex™ G-25 columns or with commercially available prepacked columns (Poly-Pack cartridge, PD-10 columns or equivalent). Purify the product by RP-HPLC if necessary.

### Conjugation of other amino-containing molecules

Virtually, any molecule containing an exposed free primary amine can be labelled with IRIS active esters. Optimal conditions should be performed by operators, which must consider these facts:

- The amino containing molecule should be dissolved at the maximum concentration possible. For aqueous buffers, the pH must be between pH 8-9.
- At basic pH, the hydrolysis occurs more slowly than at acidic pH or in distilled water. Avoid buffers containing amines (e.g. Tris buffers) or sodium azide. Best, where possible, is to dissolve the biomolecule in dry organic solvents such as DMF or DMSO.
- If it is not possible, dissolve it in the conjugation buffer (0,1 M Sodium Borate pH 8.5 or similar) and use immediately. Do not exceed pH 9.
- Where possible, perform a set of conjugation reactions using increasing excess of dye. Estimate for each reaction the F/P value and choose the most appropriate for your further experiments.

## Consideration about purification step

In most cases, e.g. for protein purification, a gel filtration step (on Sephadex™ G-25, or G-50 or equivalent ) is sufficient for removing the excess of unconjugated dye.

Alternatively, purification by microconcentration is also possible. In this case, add two or more washing steps to original protocols, to remove all traces of unincorporated dye.

In some instances, purification by reverse-phase HPLC or FPLC is preferred (e.g. for oligonucleotides purification). When working with silica-based columns, be sure to use well-capped columns.
This is because active ester present in the sample may react with silica and damage the column.

## Estimation of Dye-to-protein (D/P) ratio

For the estimation of the D/P ratio, you should know the extinction coefficient at 280 nm of your protein of interest.

For IgG, it can be assumed 170,000 $M^{-1}cm^{-1}$. Then you should measure the absorbance of your purified conjugate at 280 nm and at the maximum of the IRIS™ Dye -NHS used.
The F/P ratio is calculated with the formula:

$$D/P = A_{dye}E_{prot}/[(A_{280}-0.1A_{dye})E_{dye}]$$

Where:
$A_{dye}$ is the dye absorbance at absorption maximum.
$A_{280}$ is the absorbance at 280 nm.
$E_{dye}$ is the extinction coefficient of the IRIS™ Dye-NHS
$E_{prot}$ is the extinction coefficient of the protein
The factor 0.1 is the contribution of the dye to the absorption at 280 nm (about 10% of the absorption at its maximum

## Legal



## Contact information

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