CYANAGEN

Reagents for Molecular Biology

NEW

THE RIGHT LIGHT

SMART

Antibody Labeling Kits

012

www.cyanagen.com

About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development and production of reagents for molecular diagnostic since 2003 and one of the leading companies in the field of reagents for Western blotting and Elisa.
The main product lines are focused on chemiluminescence and fluorescent dyes for biological analysis, genomics, proteomics and chemical sensors.
They are based on Cyanagen internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
The products are extremely versatile and perfectly suited to the latest analytical instrumentation. These products are also available as OEM.

Cyanagen s.r.l. has a certified Quality System

ISO 9001-2008 QUALITY CERTIFIED

Product manual

SMART

Antibody Labeling Kit

770

Application Protocol

SMART-ANTIBODY LABELING KIT IS INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.

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Kit content

- SMART 770 - Dye: 1 or 2 vial containing the dye (each vial of reactive dye provided in the kit is sufficient for labeling 1 mg Ab)
- Labeling buffer (300μL)
- Empty Gel filtration column
- Purification resin (25 mL)
- Elution buffer (25 mL)
- Product Manual

Maximum Absorption: 774 nm
Maximum Emission: 790 nm
Molar Extinction Coefficient: 270000 M^{-1} cm^{-1}

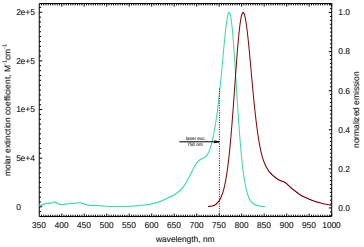
The product is shipped at ambient temperature in a sealed aluminium pack with desiccant bag.

Warnings

For research use only.
The dye is deeply coloured: care and use of gloves and suitable protective clothing to handle the vials is recommended.

Introduction

The SMART 770 - Antibody Labeling Kit is supplied with the SMART 770 - Dye, a highly efficient fluorescent molecule ideal for accurate bioanalytical measurements.
The absorption and emission spectra of the dye are reported in the following plot:



Even if validated for antibodies, the kits can be used as well as for any molecule containing a primary amino group, like peptides/proteins or 5’-aminomodified DNA oligomers and cDNA containing aminoallyl-dU-units.

1 - Dye solution preparation

Let the kit to warm at room temperature.

2 - Antibody preparation

Each SMART 770 – Dye is designed to label 1 mg of IgG (M.W. 150000) at 1 mg/mL concentration.
The antibody must be dissolved in amine free buffer. If the antibody is in an amino containing buffer, replace the buffer with PBS by dialysis.
Dilute the antibody (solid or in buffer solution) to 1 mg/mL with PBS 0.1 M pH 7.4, then add 100 µL of labeling buffer to 1 mL of antibody solution.
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3 - Conjugation procedure

Add the antibody solution (from step 2) to the vial containing the dye (SMART 770 – Dye).
Cap the vial, gently mix (do not vortex) and incubate the solution at room temperature in the dark for 1 hour, kindly shaking every 15 minutes.

4 - Isolation of the conjugate

Prepare 200 ml of 1X Elution buffer by diluting 20 ml of Elution buffer in deionized water.
Add 15 mL of Purification resin to the empty column and decant the buffer from the top until the 10 cm height is reached.
Add 10 mL of the 1X Elution buffer. Flow will automatically stop. There is no need to worry about the column drying out.
Carefully transfer the ab-labeling mixture to the top of the column and allow the solution to enter the packing.
Add 3 or 4 mL of the 1X Elution buffer. A faster moving band of labeled antibody will separate from the unconjugated dye.
When faster band arrives to the end of the column, add an additional 2.5 mL of 1X Elution buffer to the top of the column and collect the faster moving band in a clean tube. The labeled antibody should be entirely eluted by the 2.5 mL of buffer and collected in a single tube.
Add 10 ml of 1X Elution buffer to remove the excess of free dye from the column.
Stock the column at +4°C for re-use in the next reaction.

5 - Determination of Degree of Labeling (DOL)

The efficiency of the labeling may be calculated by measuring the absorbance of the antibody-dye conjugate at 280 nm (A_{280}) and at the λ_{max} of the fluorophore (A_{max}).

First, dilute the conjugate to obtain an absorbance within the 0.15-0.5 range (concentration about 0.1-0.3 mg/mL). Simply record the UV-Vis spectrum in a quartz cell with 1 cm path length. The concentration of the bound dye is

$$C_{dye} = \frac{A_{max}}{\varepsilon_{max}}$$

where ε_{max} is the molar extinction coefficient of the dye. The antibody absorbance A_{280} must be corrected because of the absorption of the dye at 280 nm, so the concentration of the antibody is

$$C_{Ab} = \frac{A_{280} - A_{max} \times CF_{280}}{\varepsilon_{Ab}}$$

where ε_{Ab} (~ 203000 $M^{-1}cm^{-1}$) is the molar extinction coefficient of the IgG and CF_{280} is the correction factor for the SMART 770 - Dye (= 0.05) given by the ratio A_{280}/A_{max} for free dye.
The Degree of Labeling can be thus calculated:

$$\frac{C_{dye}}{C_{Ab}} = \frac{A_{max} \times \varepsilon_{Ab}}{[A_{280} - (A_{max} \times CF_{280})] \times \varepsilon_{dye}}$$

Storing and Handling the Conjugates

Store the labeled antibody, which will be in PBS pH 7.4 containing 0.01% of sodium azide as preservative, at +2-6°C, and protect from light. If the final concentration of the purified antibody is less than 1 mg/mL, add bovine serum albumin (BSA) or other stabilizing protein to a final concentration 1-10 mg/mL.
At +4°C the conjugate is stable at least 3 months.
For long-term storage, divide the solution into small aliquots and freeze at -20°C.
Avoid repeated freezing and thawing.
Protect from light.
After storage it is a good practice to centrifuge solutions of conjugates in a micro centrifuge before use; use only the surnatant in the experiment in order to remove any aggregates that may be formed during storage.

Troubleshooting

Under-labeling

- antibody buffer solution contains primary amines contaminants: dialyze versus the desired buffer;
- pH of the conjugation solution too low: add more labelling buffer to raise the value to 8.3;
- different antibodies may react at different rates: optimize the labeling by changing reaction time and/or amount of dye.

Over-labeling

If the DOL is higher than the expected, next time try to:
- increase the amount of antibody (step 2), or
- decrease the reaction time in the step

Antibody was not labeled

Contact Cyanagen s.r.l.

For further information, visit **www.cyanagen.com**
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