

**CYANAGEN**  
Reagents for Molecular Biology

**NEW**

THE RIGHT LIGHT

**SMART**

Antibody Labeling Kits

**012**

[www.cyanagen.com](http://www.cyanagen.com)

# About us

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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

### 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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# 1. Introduction

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The SMART - Antibody Labeling Kit is supplied with the relative SMART - Dye, a highly efficient fluorescent molecule ideal for accurate bioanalytical measurements.

SMART - Antibody labeling kit provide a convenient tool for labeling 1 mg of antibody with large molecular weight. The kit provides everything needed for the reaction and the purification of the conjugate. Even if optimized 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.

## Warnings

The dye is deeply colored: care and use of gloves and suitable protective clothing to handle the vials is recommended.

## SMART – Antibody labeling kit product line

<b>SMART – Antibody labeling kit</b>	<b>Cod.</b>	<b>CF280</b>	<b>Abs<sub>max</sub> (H2O)</b>	<b>Em<sub>max</sub> (H2O)</b>	<b>ε (M<sup>-1</sup>cm<sup>-1</sup>) (H2O)</b>
SMART 488 - Antibody labeling kit	F5A119N	0.11	494	520	740000
SMART 550 - Antibody labeling kit	F4D091N	0.08	550	565	150000
SMART 645 - Antibody labeling kit	F2H085N	0.05	648	667	250000
SMART 770 - Antibody labeling kit	F1L092N	0.05	774	790	270000

## 2. Components and storage

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### Kit components

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

### Storage

Store SMART - Dye at -20°C and the other components at +4°C

## 3. Dye preparation

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Allow the kit to warm up to room temperature.

## 4. Antibody preparation

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Each SMART - 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, remove the buffer by dialysis. Dilute the antibody (solid or in buffer solution) to 1 mg/mL with 1X PBS pH 7.4, then add 100  $\mu$ L of labeling buffer to 1 mL of antibody solution.

## 5. Conjugation procedure

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Add the antibody solution (from step 4) to the vial containing the dye (SMART - 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.

## 6. Isolation of the conjugate

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Prepare 200 ml of 1X Elution buffer by diluting 20 ml of SMART - Elution buffer in deionized water.

Add 15 mL of SMART- 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.

## 7. Determination of Degree of Labeling (DOL)

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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  $\lambda_{max}$  of the fluorophore ( $A_{max}$ ).

The concentration of the bound dye is

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

where  $\epsilon_{max}$  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

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

where  $\epsilon_{Ab}$  is the molar extinction of the antibody (i.e 203000 for IgG) and  $CF_{280}$  is the correction factor for the SMART - Dye given by the ratio  $A_{280}/A_{max}$  for free dye.

$\epsilon_{max}$  and  $CF_{280}$  for each dye are reported in table at page 4.

The Degree of Labeling can be thus calculated:

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

**Note:** The reported extinction molar coefficients are valid for a 1 cm pathlength. For different pathlengths, the concentration must be divided by the pathlength in cm.

## 8. Storing and Handling the Conjugate

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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 supernatant in the experiment in order to remove any aggregates that may be formed during storage.



## 9. Troubleshooting

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### **Under-labeling**

- antibody buffer solution contains primary amines contaminants: dialyze versus the desired buffer;
- pH of the conjugation solution too low: add more labeling 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

### **Insufficient removal of free dyes**

-Remove the free dye by applying the conjugate to another column or by extensive dialysis.

### **Antibody was not labeled**

Contact Cyanagen at [technical.support@cyanagen.com](mailto:technical.support@cyanagen.com)

## 10. Ordering information

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<b>Product Description:</b>	<b>Sufficient For:</b>	<b>Order-No:</b>
SMART 488 - Antibody labeling kit	1 mg of antibody	F5A119N,CLK01
	2 x 1 mg of antibody	F5A119N,CLK02
SMART 550 - Antibody labeling kit	1 mg of antibody	F4D091N,CLK01
	2 x 1 mg of antibody	F4D091N,CLK02
SMART 645 - Antibody labeling kit	1 mg of antibody	F2H085N,CLK01
	2 x 1 mg of antibody	F2H085N,CLK02
SMART 770 - Antibody labeling kit	1 mg of antibody	F1L092N,CLK01
	2 x 1 mg of antibody	F1L092N,CLK02

For further information visit [www.cyanagen.com](http://www.cyanagen.com)

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