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 versatie 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 Micro

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.

www.cyanagen.com

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1. Introduction

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

SMART Micro – Antibody labeling kit provide a convenient tool for labeling small amounts of antibody, from 50 to 100 μg. 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 Micro – Antibody labeling kit product line

SMART Micro – Antibody labeling kit	Cod.	CF280*	Abs_{max} (H2O)	Em_{max} (H2O)	ε** (H2O)
SMART Micro 488 – Antibody labeling kit	F5A122N	0.11	494	520	74000
SMART Micro 550 – Antibody labeling kit	F4D123N	0.08	550	565	150000
SMART Micro 645 – Antibody labeling kit	F2H124N	0.05	648	667	250000
SMART Micro 770 – Antibody labeling kit	F1L125N	0.05	774	790	270000

*Correction factor (A_{280}/A_{max})
**Molar extinction coefficient ($M^{-1}cm^{-1}$)

2. Components and storage

Kit components

- SMART Micro - Dye: 3 vials containing the dye (each vial of reactive dye provided in the kit is sufficient for labeling from 50 to 100 μg Ab)
- SMART - Labeling buffer (300 μL)
- SMART - Purification spin column (3)
- Collection tube (3)

Storage

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

3. Antibody preparation

Each SMART Micro – Dye is designed to label from 50 to 100 μg 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 containing 0.01% Sodium Azide, then add 10% of labeling buffer to the antibody solution.

4. Conjugation procedure

Allow the kit to warm up to room temperature. Add 10 μL of dH_2O to the SMART Micro – Dye and mix by pipetting up and down.
Calculate the volume of reactive dye solution to use with the following formula:

μL reactive dye to use= μg Ab x 0.05

Where μg Ab is the amount of the antibody to be labeled. This volume is calculated to get an optimal Degree of labeling of IgG with the SMART Micro – Dye. Since proteins react with fluorophores at different rate and retain biological activity at different DOL, the optimal DOL estimated for the SMART – Micro may not always result in optimal labeling. To increase or decrease the DOL, use more or less volume of reactive DYE in respect to the calculated amount with the previous equation.

Add the volume of dye solution to the pH-adjusted solution of antibody and mix by pipetting up and down. Cap the vial and incubate the reaction mixture at room temperature in the dark for 1 hour, pipetting up and down every 15 minutes.

Note: The dye solution should be prepared immediately before use and any leftover solution must be discarded.

5. Isolation of the conjugate

- Place the SMART - Purification spin column in a 2 mL Collection Tube.
- Centrifuge at *1,000 x g* for 2 minutes.
- Transfer the spin column to a 1,5 mL microcentrifuge tube.
- Carefully load the sample (20-100 μL) onto the center of the gel bed surface.
- Centrifuge again at *1,000 x g* for 3 minutes.
- The purified sample can be recovered at the bottom of the 1,5 mL microcentrifuge tube (approximately the same volume as the loaded sample).

6. 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}).

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} 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.

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

The Degree of Labeling can be thus calculated:

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

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

7. Storing and Handling the Conjugate

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.

8. Troubleshooting

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 dyalisis.

Antibody was not labeled

Contact Cyanagen at technical.support@cyanagen.com

9. Ordering information

Product Description:	Sufficient For:	Order-No:
SMART Micro 488 – Antibody labeling kit	3 x 50 - 100 µg of antibody	F5A122N,CLK001
SMART Micro 550 – Antibody labeling kit	3 x 50 - 100 µg of antibody	F4D123N ,CLK001
SMART Micro 645 – Antibody labeling kit	3 x 50 - 100 µg of antibody	F2H124N,CLK001
SMART Micro 770 – Antibody labeling kit	3 x 50 - 100 µg of antibody	F1L125N,CLK001

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

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CYANAGEN
Reagents for Molecular Biology