

CHROMIS 550 ANTIBODY LABELING KIT

Application Protocol

KIT CONTENT

- **CHROMIS 550 Dye:** 1 vial containing the dye. (optimal amount for 1 mg of antibody)
Maximum Absorption: 553 nm
Maximum Emission: 568 nm
Molar Extinction Coefficient: 150000 M⁻¹cm⁻¹
- **CHROMIS XR:** 1 vial containing 70 µL solution in dry DMF

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

Upon receipt store at -20 °C
Shipped at ambient temperature
Stability: at least four months

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.
The CHROMIS XR is moisture and temperature sensitive: keep cold and open only before use.

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INTRODUCTION

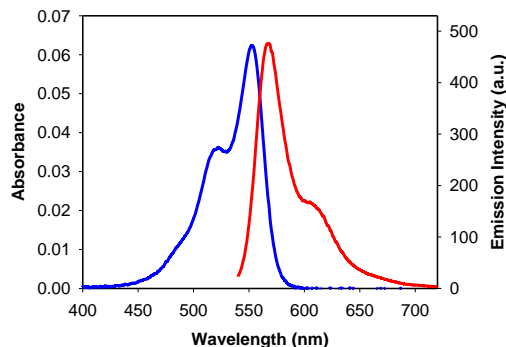
To meet the ever growing demand of fluorescent probes for bioanalytical applications and biological techniques Cyanagen has created **CHROMIS LABELING KIT**, a line of conceptually new kits for the labeling of biomolecules.

The **CHROMIS 550 Antibody Labeling Kit** was specifically developed to offer ideal conditions for antibody labeling. The amount of dye for each kit is tuned to give an optimal 5-10 dye-to-protein ratio for 1 mg of total target protein.

The innovative two-vial formulation has many advantages:

- Dye purity: the reactive dye is generated in situ thus affording the maximum efficiency in the conjugation step.
- Easy-to-use formulation: the reaction is carried out mixing the supplied reagents and adding the target antibody.
- Extended dye stability: when properly stored kits have a stability of more than four months.

The **CHROMIS 550 Antibody Labeling Kit** is supplied with the **CHROMIS 550 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:



1. DYE SOLUTION PREPARATION

Let the kit to warm at room temperature. Add 50 µL of the **CHROMIS XR** solution to the **CHROMIS 550 Dye** vial. Cap the vial, dissolve the dye by vortexing and keep in the dark at room temperature for 30 minutes.

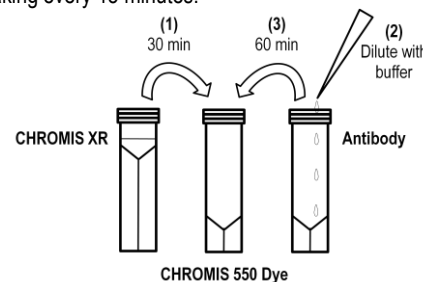
2. ANTIBODY PREPARATION

Each kit is designed to label 1 mg of IgG (M.W. 150000) at 1 mg/mL solution. The antibody must be dissolved in amine free buffer.

- **For antibody buffer solution:** dilute to 1 mg/mL and then add 100 µL of 1 M sodium bicarbonate buffer.
- **For solid antibody:** dilute 1 mg of antibody by adding 1 mL of 0.1 M sodium bicarbonate buffer.

3. CONJUGATION PROCEDURE

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



4. ISOLATION OF THE CONJUGATE

Commonly employed methods use gel permeation chromatography (on Sephadex™ G25) to separate the conjugated product from unreacted dye: equilibrate the column with PBS or desired buffer and elute using the same buffer in order to collect the first coloured band. Alternatively use commercially available spin columns. Less efficient separation may be also achieved by dialysis or ultrafiltration.

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 fluorophor (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

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

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

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

where ϵ_{Ab} (~ 210000 M⁻¹cm⁻¹) is the molar extinction coefficient of the IgG and CF_{280} is the correction factor for the **CHROMIS 645 Dye** (= 0.080) given by the ratio A_{280} for free dye/ A_{max} for free dye.

The Degree of Labeling can be thus calculated:

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

TROUBLESHOOTING

Under-labeling

- antibody buffer solution contains primary amines contaminants: dialyze versus the desired buffer;
- pH of the conjugation solution too low: add more bicarbonate 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 3, or
 - add small portions of the dye solution (from step 1) to the antibody until an optimal DOL is obtained.

Antibody was not labelled

Preparation of the Dye Solution in the step 1 did not afford the active dye: Contact Cyanagen s.r.l.