Chromeo™ 488, 494, 546, and 642 Antibody Labeling Kits

(version B1)

Chromeo™ 488 Antibody Labeling Kit (Catalog No. 15090)
Chromeo™ 494 Antibody Labeling Kit (Catalog No. 15091)
Chromeo™ 546 Antibody Labeling Kit (Catalog No. 15092)
Chromeo™ 642 Antibody Labeling Kit (Catalog No. 15093)

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Introduction

Active Motif's Antibody Labeling Kits provide a convenient means to label any immunoglobulin or protein with a fluorescent Chromeo™ Dye. Chromeo Dyes exhibit superior fluorescent properties with excitability between 450 and 650 nm, fluorescence emission between 510 and 680 nm (Table 1), Stokes shifts between 16 and 124 nm and tolerance of pH and limited photobleaching. The dyes are supplied as *N*-hydroxysuccinimide (NHS) ester reactive amines that covalently label amino groups found on other biomolecules (peptides, proteins, and immunoglobulins).

Chromeo™ Dye	Excitation/Emission (nm)	Dyes of similar spectra
Chromeo™ 488	490/527	FITC, Alexa® 488
Chromeo™ 494	500/628	
Chromeo™ 546	549/570	Cy3®
Chromeo™ 642	646/666	Cy5®

Table 1: Approximate excitation and emission maxima of typical Chromeo™-IgG conjugates.

Each Antibody Labeling Kit contains three vials of Chromeo dye (NHS-Ester); each vial is sufficient to label 1 mg of IgG (or a similar amount of other proteins). Each kit includes Coupling Buffer, three purification columns with purification resin and PBS Elution Buffer. All reagents can be stored long term at 4°C except the Chromeo dye (NHS-Ester), which must be stored at -20°C. All reagents are guaranteed stable for 6 months when stored properly.

Note: Chromeo NHS-Ester Dyes are very sensitive to moisture, so should be stored in the dark in the original packaging until use. To avoid a decrease in activity due to moisture condensation, the vial should be slowly brought to room temperature before opening. Prepare the dye solution immediately before use and do not store any remaining stock solution.

product	format	catalog no.
Chromeo™ 488 Antibody Labeling Kit	1 kit	15090
Chromeo™ 494 Antibody Labeling Kit	1 kit	15091
Chromeo™ 546 Antibody Labeling Kit	1 kit	15092
Chromeo™ 642 Antibody Labeling Kit	1 kit	15093

Kit Performance

Chromeo™ Antibody Labeling Kits are for research use only. Not for use in diagnostic procedures.

Chromeo 488 NHS-Ester Antibody Labeling Kit

The Chromeo 488 NHS-Ester Antibody Labeling Kit labels antibodies and proteins with a bright fluorescent signal that has its peak at 527 nm and an excitation range of 470 to 500 nm.

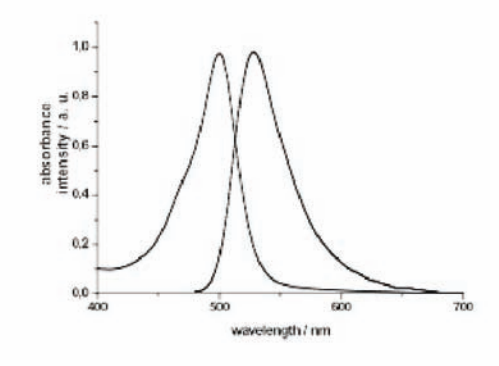


Figure 1: Goat anti-rabbit IgG labeled with 488 NHS-Ester using the Chromeo™ 488 NHS Ester Antibody Labeling Kit. 2.5 moles of dye were conjugated per mole of antibody.

Chromeo 494 NHS-Ester Antibody Labeling Kit

The Chromeo 494 NHS-Ester Antibody Labeling Kit is used to label antibodies and proteins with a broad Stokes shift with its peak fluorescent emission at 628 nm and an excitation range of 480 to 550 nm.

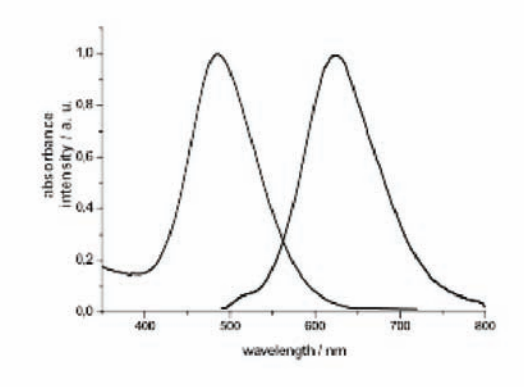


Figure 2: Goat anti-rabbit IgG labeled with 494 NHS-Ester using the Chromeo™ 494 NHS-Ester Antibody Labeling Kit. 6.3 moles of dye were conjugated per mole of antibody.

Chromeo 546 NHS-Ester Antibody Labeling Kit

The Chromeo 546 NHS-Ester Antibody Labeling Kit is used to label antibodies and proteins with a bright fluorescent signal that has its peak at 570 nm and an excitation range of 500 to 550 nm.

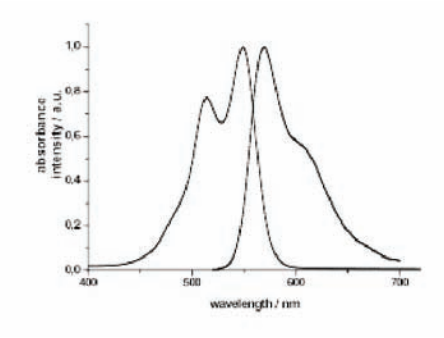


Figure 3: Goat anti-rabbit IgG labeled with 546 NHS-Ester using the Chromeo™ 546 NHS-Ester Antibody Labeling Kit. 3.9 moles of dye were conjugated per mole of antibody.

Chromeo™ 642 NHS-Ester Antibody Labeling Kit

The Chromeo 642 NHS-Ester Antibody Labeling Kit is used to label antibodies and proteins with a bright fluorescent signal that has its peak at 666 nm and an excitation range of 630 to 650 nm.

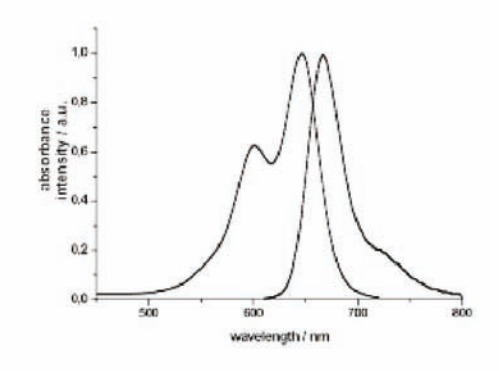


Figure 4: Goat anti-rabbit IgG labeled with 642 NHS-Ester using Chromeo™ 642 NHS-Ester Antibody Labeling Kit. 1.7 moles of dye were conjugated per mole of antibody.

Kit Components and Storage

Please store each component at the temperature indicated in the table below.

Reagents	Quantity	Storage / Stability
1 M $NaHCO_3$ (Coupling Buffer)	200 µl	4°C to -20°C
DMF	200 µl	4°C
10X Elution Buffer	10 ml	4°C to -20°C
Purification resin in 1X PBS	42 ml	4°C
Assembled purification columns*	3	Room temperature
Polyethylene funnels	3	Room temperature
Chromeo™ NHS-Ester	3 vials	-20°C

*Assembled columns include polyethylene column, porous bed support and outlet cap.

Additional materials required

- Distilled water
- Column support stand
- Collection tube
- Beaker
- Spectrophotometer

Reagent Preparation

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Prior to starting the assay, please prepare the following:

Elution Buffer

Prepare the required amount of Elution Buffer by diluting the 10X Elution Buffer 1:10 in distilled water. Typically, less than 10 ml will be required for each purification.

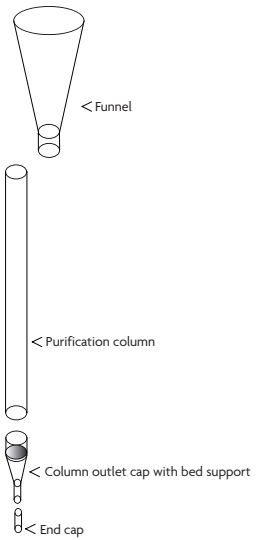
Antibody to be Labeled

For labeling reactions we recommend an optimal antibody concentration of 2 mg/ml.

The buffer the antibody or protein is formulated in should not contain any primary amines or ammonium ions (*e.g.* Tris buffers or glycine). Sodium azide concentrations below 3 mM will not affect labeling efficiency. However, if the buffer that the antibody or protein is dissolved in contains these components or if it is greater than 3 mM sodium azide, the buffer must be replaced with 1X PBS by buffer exchange or dialysis.

Column Assembly

Columns are provided pre-assembled with separate polyethylene funnels.



Note: This protocol has been optimized for labeling antibodies. Because antibodies differ in activity and functional properties, some may react more quickly than others in the conjugation process. Also, some antibodies may retain function after labeling better than others due to their structure. Because antibodies react differently and can be used in a wide variety of applications, this labeling protocol may have to be optimized depending on the antibody or the application. Please refer to the troubleshooting section if poor labeling occurs, or if your initial results are not optimal.

Step 1: Antibody Labeling Procedure

1. Allow the 1 M NaHCO3 (Coupling Buffer) to warm to room temperature before starting. Add 50 µl of room temperature 1 M NaHCO3 (Coupling Buffer) to 500 µl of your 2 mg/ml antibody solution.
2. Refer to the Certificate of Analysis for how much DMF to add to the vial containing the lyophilized Chromeo™ NHS-Ester reactive dye and mix gently with pipette tip.
3. Refer to the Certificate of Analysis for the amount of reactive dye/DMF mixture to transfer to the antibody solution (from No. 1 above) then mix thoroughly.
4. Incubate for 1 hour with shaking at room temperature. During this incubation step, assemble the purification column as described in the next step.

Step 2: Purification of Labeled Antibody

1. Assemble the purification column by positioning it upright and removing the end cap. Attach a funnel to the top of the column. Shake the closed bottle with the purification resin thoroughly to ensure a homogenous suspension. Pipet the resin onto the column bed, allowing excess buffer to drain away into a small beaker. Resin should be packed into the column until the resin is 3 cm from the top of the column. Make sure the resin is thoroughly mixed before pipetting additional resin onto the column. Approximately two 7 ml volumes of the resin slurry are needed to pack the column.

   Note: Allow excess buffer to drain into the column. Watch to make sure the buffer flows through with an even flow. If the buffer flow is slow, stops before empty or changes rate, simply remove the resin, resuspend the mixture and add back to the column. Make sure that the column is not clamped to the support so tightly that buffer flow is obstructed.

2. After the 1 hour incubation is complete, carefully load the reaction mixture evenly onto the column. The funnel portion of the column may be removed before loading the sample. Allow the reaction mixture to enter the column resin.

3. Rinse the reaction vial with 100 µl of 1X Elution Buffer, then apply this to the column. Allow the solution to enter the column. As the reaction mixture runs through the column, two bands of color will be apparent. You will collect the first colored band, which contains the labeled antibody, into a small vessel by adding Elution Buffer to the column as necessary. Do not collect the slower moving band, which is unincorporated dye.

4. Slowly add 1X Elution Buffer, taking care not to disturb the column bed. Continue adding Elution Buffer until first band containing the labeled antibody has been eluted. Depending on the size of the protein being conjugated, the bands may elute quickly. An antibody will typically begin to elute after the addition of approximately 2 ml, and will be collected in a volume of approximately 1 to 2 ml.

   Note: Collect all of the Elution Buffer if you want to check for or recover any unconjugated antibody or protein, as this will run off before the first band. (There is no benefit to collecting the 2nd band, as this is unincorporated dye.)

5. Check the concentration of the antibody conjugate. If the concentration of the conjugate is below 1 mg/ml, add BSA to a final concentration of 1 to 5 mg/ml to ensure stability of the protein. Store the conjugated protein at 2-6°C and protect from light. For long-term storage, aliquot the fluorescent conjugate and store the aliquots frozen at -20°C or lower. Avoid subjecting the conjugate to repeated freezing and thawing.

Step 3: Determining the Degree of Labeling

Chromeo™ 488 NHS-Ester

1. Measure the absorbance of the labeled antibody solution at 280 nm and 490 nm (A_{280} and A_{490}) in a cuvette with 1 cm path length. Dilution of the solution may be necessary.
2. Calculate the concentration of antibody in the sample:

$$\text{Antibody concentration (M)} = \frac{[A_{280} - (A_{490} \times 0.16)] \times \text{dilution factor}}{210{,}000}$$

   210,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of a typical IgG and 0.16 is a correction factor to account for absorption of the dye at 280 nm. Non-IgG proteins will likely have significantly different molar extinction coefficients.

3. Calculate the degree of labeling:

$$\text{Moles of dye per mole of antibody} = \frac{A_{490} \times \text{dilution factor}}{73{,}000 \times \text{antibody concentration (M)}}$$

   73,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of the Chromeo 488 dye at 490 nm.

Chromeo™ 494 NHS-Ester

1. Measure the absorbance of the labeled antibody solution at 280 nm and 485 nm (A_{280} and A_{485}) in a cuvette with 1 cm path length. Dilution of the solution may be necessary.
2. Calculate the concentration of antibody in the sample:

$$\text{Antibody concentration (M)} = \frac{[A_{280} - (A_{485} \times 0.11)] \times \text{dilution factor}}{210{,}000}$$

   210,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of a typical IgG and 0.11 is a correction factor to account for absorption of the dye at 280 nm. Non-IgG proteins will likely have significantly different molar extinctions coefficients.

3. Calculate the degree of labeling:

$$\text{Moles of dye per mole of antibody} = \frac{\text{A485} \times \text{dilution factor}}{55{,}000 \times \text{antibody concentration (M)}}$$

   55,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of the Chromeo 494 dye at 485 nm.

Chromeo™ 546 NHS-Ester:

1. Measure the absorbance of the labeled antibody solution at 280 nm and 549 nm (A_{280} and A_{549}) in a cuvette with 1 cm path length. Dilution of the solution may be necessary.
2. Calculate the concentration of antibody in the sample:

$$\text{Antibody concentration (M)} = \frac{[A_{280} - (A_{549} \times 0.09)] \times \text{dilution factor}}{210{,}000}$$

210,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of a typical IgG and 0.09 is a correction factor to account for absorption of the dye at 280 nm. Non-IgG proteins will likely have significantly different molar extinction coefficients.

3. Calculate the degree of labeling:

$$\text{Moles of dye per mole of antibody} = \frac{A_{549} \times \text{dilution factor}}{96{,}800 \times \text{antibody concentration (M)}}$$

96,800 $cm^{-1}M^{-1}$ is the molar extinction coefficient of the Chromeo 546 dye at 549 nm.

Chromeo™ 642 NHS-Ester:

1. Measure the absorbance of the labeled antibody solution at 280 nm and 646 nm (A_{280} and A_{646}) in a cuvette with 1 cm path length. Dilution of the solution may be necessary.
2. Calculate the concentration of antibody in the sample:

$$\text{Antibody concentration (M)} = \frac{[A_{280} - (A_{646} \times 0.027)] \times \text{dilution factor}}{210{,}000}$$

210,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of a typical IgG and 0.027 is a correction factor to account for absorption of the dye at 280 nm. Non-IgG proteins will likely have significantly different molar extinction coefficients.

3. Calculate the degree of labeling:

$$\text{Moles of dye per mole of antibody} = \frac{A_{646} \times \text{dilution factor}}{180{,}000 \times \text{antibody concentration (M)}}$$

180,000 $cm^{-1}M^{-1}$ is the molar extinction coefficient of the Chromeo 642 dye at 646 nm.

Section B. Troubleshooting Guide

Problem/question	Possible Cause	Recommendation
Antibody did not get labeled	The antibody was in an incompatible buffer that contained primary amines (like Tris or glycine), ammonium ions or > 3 mM sodium azide.	Dialyze antibody into 1X PBS before conjugation.
	The NHS-Ester hydrolyzed and became non-reactive.	Prepare the dye solution immediately before use and do not store any remaining stock solution.
Inefficient labeling	Antibody or protein was too dilute.	Try to concentrate the starting material.
	Antibody or protein is in a buffer with a low pH.	The optimal pH for NHS-Esters is between pH 8.1 to 8.3; try to reach the pH range by doubling the amount of Coupling Buffer, or replace the low pH buffer by exchange or dialysis.
Over-labeling	Not enough protein to label or reaction time too long.	Add more protein to the reaction or decrease the reaction time.

Section C. Related Products

Fluorescent Dyes	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Carboxylic Acid	488 nm / 517 nm	1 mg	15510
Chromeo™ 488 NHS-Ester	488 nm / 517 nm	1 mg	15511
Chromeo™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromeo™ 494 Carboxylic Acid	494 nm / 628 nm	1 mg	15110
Chromeo™ 494 NHS-Ester	494 nm / 628 nm	1 mg	15111
Chromeo™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromeo™ 505 Carboxylic Acid	505 nm / 526 nm	1 mg	15610
Chromeo™ 505 NHS-Ester	505 nm / 526 nm	1 mg	15611
Chromeo™ 546 Carboxylic Acid	545 nm / 561 nm	1 mg	15210
Chromeo™ 546 NHS-Ester	545 nm / 561 nm	1 mg	15211
Chromeo™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromeo™ 642 Carboxylic Acid	642 nm / 660 nm	1 mg	15310
Chromeo™ 642 NHS-Ester	642 nm / 660 nm	1 mg	15311
Chromeo™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

Fluorescent Secondary Antibodies	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042
Chromeo™ 546 Goat anti-Mouse IgG	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	1 mg	15043
Chromeo™ 642 Goat anti-Mouse IgG	1 mg	15034
Chromeo™ 642 Goat anti-Rabbit IgG	1 mg	15044

Fluorescent Cell Stain	Format	Catalog No.
LavaCell™	200 µg	15004

Fluorescent Protein Labeling	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

Luciferase Assays	Format	Catalog No.
RapidReporter™ Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter™ pRR-High vector	10 µg	33003
RapidReporter™ pRR-High Assay	100 rxns	33004
RapidReporter™ pRR-Low vector	10 µg	33005
RapidReporter™ pRR-Low Assay	100 rxns	33006
RapidReporter™ pRR-High-CRE vector	10 µg	33007
RapidReporter™ pRR-High-CRE Assay	100 rxns	33008
RapidReporter™ pRR-High-GR vector	10 µg	33011
RapidReporter™ pRR-High-GR Assay	100 rxns	33012
RapidReporter™ pRR-High-IRF-1 vector	10 µg	33017
RapidReporter™ pRR-High-IRF-1 Assay	100 rxns	33018
RapidReporter™ pRR-High-NFκB vector	10 µg	33009
RapidReporter™ pRR-High-NFκB Assay	100 rxns	33010
RapidReporter™ pRR-High-STAT1 vector	10 µg	33015
RapidReporter™ pRR-High-STAT1 Assay	100 rxns	33016
RapidReporter™ pRR-High-STAT3 vector	10 µg	33013
RapidReporter™ pRR-High-STAT3 Assay	100 rxns	33014

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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