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Instruction Manual

Dye 488 Antibody Labelling Kit

(Kat.-Nr./cat. no. 59000)

Dye 550 Antibody Labelling Kit

(Kat.-Nr./cat. no. 59001)

Dye 645 Antibody Labelling Kit

(Kat.-Nr./cat. no. 59002)

Dye 770 Antibody Labelling Kit

(Kat.-Nr./cat. no. 59003)

Content

Instruction Manual

General information

The Antibody Labelling Kit is supplied with a highly efficient fluorescent molecule ideal for accurate bioanalytical measurements. The kit is a convenient tool for labelling 1 mg of antibody with large molecular weight.
It includes everything needed for the labelling 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'-amino-modified DNA oligomers and cDNA containing amino-allyl-dU-units.

Warnings

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

Spectral properties

Antibody Labelling Kit	Cat. no.	CF_{280}	Abs_{max} (H_2O)	Em_{max} (H_2O)	ε ($M^{-1}cm^{-1}$) (H_2O)
Dye 488	59000	0.11	494	520	74000
Dye 550	59001	0.08	550	565	150000
Dye 645	59002	0.05	648	667	250000
Dye 770	59003	0.05	774	790	270000

Tab. 1: Spectral properties of the different dyes

Kit components

- Dye: 1 or 2 vials
  Each vial of reactive dye provided in the kit is sufficient for labelling 1 mg Ab
- 300 µl Labelling buffer
- Empty Gel filtration column
- 25 ml Purification resin
- 25 ml 10x Elution buffer

Storage conditions

Kit component	Recommended storage temperature
Dye	-15 °C to - 25 °C
Labelling buffer	+2 °C to + 8 °C
Purification resin	+2 °C to + 8 °C
10x Elution buffer	+2 °C to + 8 °C

Protocol

- **Dye preparation**
  Allow the kit to warm up to room temperature

- **Antibody preparation**
  Each dye is designed to label 1 mg of IgG (MW 150,000) 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) to1 mg/ml with1x PBS pH 7.4 and add 100 µl of labelling buffer to1 ml of antibody solution.

- **Labelling/Conjugation procedure**
  Add the antibody solution (see **Antibody preparation**) to the vial containing the 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.

- **Isolation of the labelled antibody (conjugate)**

  (1) Prepare 200 ml of 1x Elution buffer
  20 ml of 10x Elution buffer add 200 ml with dist. water.

  (2) Add 15 ml Purification resin to the empty column and decant the buffer from the top until the 10 cm height is reached.

  (3) Add 10 ml of the 1x Elution buffer.
  Flow will automatically stop.
  There is no need to worry about the column drying out.

  (4) Carefully transfer the Ab-labelling mixture to the top of the column and allow the solution to enter the packing.

  (5) Add 3 or 4 ml of the 1x Elution buffer.
  A faster moving band of labelled antibody will separate from the free/unbound dye.

  (6) 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 labelled antibody should be entirely eluted by the 2.5 ml of buffer and collected in a single tube.

  (7) Add10 ml of 1x Elution buffer to remove the excess of free dye from the column.

  (8) Store the column at + 2 °C to + 8 °C for re-use in the next reaction.

Determination of Degree of Labelling (DOL)

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

The concentration of the bound dye

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

ε_{max}: molar extinction coefficient of the dye

The antibody absorbance A_{280} must be corrected because of the absorption of the dye at 280 nm.

The concentration of the antibody

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

ε_{Ab}: molar extinction of the antibody, i.e. 203000 for IgG and

CF_{280}: correction factor for the dye given by the ratio A_{280} / A_{max} for free dye.

ε_{max} and CF_{280} for each dye are given in Tab. 1 (Spectral properties of the different dyes).

Calculation of the DOL

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

Note:
The given molar extinction coefficients are valid for a 1 cm-path length.
For different path lengths, the concentration must be divided by the path length in cm.

Storage and Handling of the labelled antibody

Store the labelled antibody, which will be in PBS pH 7.4 containing 0.01% of sodium azide as preservative, light protected at +2 °C to +8 °C.

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 he recommended storage temperature, the conjugate will be stable at least 3 months.

For long-term storage, make small aliquots of the solution and freeze at - 25 °C to - 15 °C.

Avoid repeated freezing and thawing. Protect from light.

Prior use, after storage and thawing, it is a good practice to centrifuge solutions of conjugates in a micro centrifuge.

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

Troubleshooting

- **Under-labelling**
  - 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 pH to 8.3
  - Different antibodies may react at different rates:
    Optimize the labelling by changing reaction time and/or amount of dye.

- **Over-labeling**
  - If the DOL is higher than the expected:
    Increase the amount of antibody or decrease the reaction time of the a labelling step.

- **Insufficient removal of free dyes**
  - Remove the free dye by applying the conjugate to another column or by extensive dialysis.

- **Antibody was not labelled**
  - Contact SERVA: info@serva.de

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Ver 02/2020