abcam

Product datasheet

PE/Cy5.5® Conjugation Kit ab102899

1 Image

Overview

Product name

PE/Cy5.5® Conjugation Kit

Product overview

Abcam's PE/Cy5.5 Conjugation Kit provides a simple and quick process to conjugate your primary antibodies with PE/Cy5.5. PE/Cy5.5 is a tandem conjugate: PE (energy donor) has an excitation wavelength of 565nm and Cy5 (energy acceptor) has an emission wavelength of 693nm.

The conjugated antibody can be used straight away in WB, ELISA, IHC etc

Learn more about buffer compatibility, protein/secondary antibody conjugation and labelling chemistry in our FAQs.

Notes

Amount and volume of antibody

In view of the large size of the PE/Cy5.5 label, the amount of antibody used in a labeling reaction with the tandem must always be less than the amount of PE/Cy5.5, in order that the PE/Cy5.5 is in a slight molar excess. The best ratio for any new antibody reagent must be determined by experimentation but 50-60µg of IgG antibody for every 100µg of PE/Cy5.5 usually gives optimal results. 50µg of antibody corresponds to an Antibody:PE/Cy5.5 molar ratio of 1:1.

The volume in which the antibody is added ideally should be around 40µl. Where the concentration of antibody is relatively low, and where it is impractical to concentrate the antibody, up to twice the volume stated above may be added without any significant loss in conjugation efficiency.

Protocol

Before you add antibody to the PE/Cy5 mix, add 10µl of Modifier reagent for each 100µl of antibody to be labeled. Mix gently.
Remove the screw cap from the vial of PE/Cy5 mix and pipette the antibody sample (with added Modifier) directly onto the lyophilised material. Resuspend *gently* by withdrawing and re-dispensing the liquid once or twice using a pipette.

Place the cap back on the vial and leave the vial standing for 3 hours in the dark at room temperature (20-25°C). Alternatively, and often more conveniently, conjugations can be set up and left overnight, as the longer incubation time has no negative effect on the conjugate.

After incubating for 3 hours (or more), add 1µl of Quencher reagent for every 10µl of antibody used. The conjugate can be used after 30 minutes.

Storage of conjugates

For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

Sample Buffer

Ideally, the antibody to be labeled should be in 10-50mM amine-free buffer pH range 6.5 to 8.5. However, many buffers outside these limits of concentration and pH can be accommodated. Modest concentrations of Tris buffer are also tolerated.

Amine-free buffers, including MES, MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 6.5-8.5, as the addition of Modifier provides the conditions necessary for efficient conjugation. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect. If the amine-free buffer is relatively concentrated and outside the pH range 6.6-8.5 you may need to add more Modifier for each 10µl of antibody. Excess Modifier is provided so that you can check the pH of the buffer after the addition of the modifier. Ideally the pH should be around 7.3-7.6, though efficient conjugation occurs anywhere between pH 6.8 and 7.8. Avoid buffer components that are nucleophilic, as these may react with the kit chemicals. If your buffer contains primary amines (e.g. amino acids, ethanolamine) and/or thiols (e.g. mercaptoethanol, DTT), you should consider using our Concentration and Purification Kits (ab102778 or ab102784). (Note: Unusually, for an amine, Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less).

Disclaimer

Labeling of the antibody will not work if the conjugation blocks the active paratope. This situation is rare.

The antibody to be labelled should be purified, in an appropriate buffer for conjugation and at a suitable concentration, as described in the protocol booklet. If not, consider using our antibody purification and concentration kits.

Tested applications

Suitable for: Conjugation

Properties

Storage instructions

Store at -20°C. Please refer to protocols.

Components	300 µg	1 mg	100 µg	30 µg
Modifier reagent	1 vial	1 vial	1 vial	1 vial
PE/Cy5.5 mix	3 x 100µg	1 x 1mg	1 x 100µg	3 x 10µg
Quencher reagent	1 vial	1 vial	1 vial	1 vial

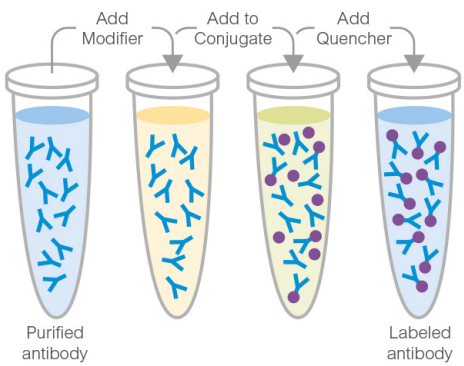
Applications

Our Abpromise guarantee covers the use of **ab102899** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Conjugation		Use at an assay dependent dilution.

Images



Conjugation

This illustration demonstrates a general procedure and will slightly vary dependent on the conjugate used.

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