



Fluoro-Trap™ Fluorescein labeling kit

711-0010

For 5 x 1mg antibody labeling reactions

Release 001; November 2006

Technical bulletin 2003

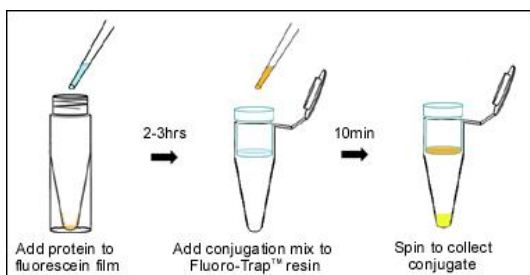
1. INTRODUCTION

The Fluoro-Trap™ Fluorescein labeling kit can be used to set up labeling reactions extremely quickly (in seconds), simply by adding a solution of the protein to be labeled to a tube coated with a thin film of reactive dye (Figure 1).

Once the conjugate has formed, Fluoro-Trap™ resin is used to remove excess free label from the conjugate, thereby circumventing the tedious desalting or dialysis steps that are required in other fluorescein labeling procedures.

Fluoro-Trap™ labeling systems can be used to label antibodies and other proteins with high recoveries and minimal sample dilution.

Fig. 1 Fluoro-Trap™ labeling system



The thin film of reactive fluorescein dye dissolves upon addition of a solution of the antibody (or other biomolecule) to be labeled. Once the conjugate has formed it is transferred to the tube insert, which contains Fluoro-Trap™ resin on a retaining membrane. The resin selectively captures excess free label from the solution and the fluorescein-labeled biomolecule is collected by briefly spinning the collecting tube in a centrifuge. The conjugate passes through the retaining membrane and the tube insert, with spent Fluoro-Trap™ resin, is removed and discarded. The hands-on time for the entire labeling procedure is about 3 minutes.

2. INSTRUCTIONS

2.1 Storage and components

The kit is shipped at ambient temperature in a tamper-evident polypropylene container. Store at 4°C upon receipt.

Kit contents:

Five tubes of Fluorescein label

Five tubes of Fluoro-Trap™ resin

1 tube of Fluoro-Trap™ reaction buffer

1 tube of Fluoro-Trap™ quencher

1 tube of Fluoro-Trap™ neutralizer

1 protocol

2.2 Considerations before use

2.2.1 Sample buffer

Ideally, the 1mg of antibody to be labeled should be in 10-50mM amine-free buffer, pH range 6.5 to 8.5. However, some amine-containing buffers can be tolerated. Appendix 1 gives further guidance on buffers and compatible additives.

2.2.2 Sample volume

For the best results, the sample volume should be 0.5-2ml, which means that the concentration of antibody ideally should be 0.5mg/ml or more.

2.3 Setting up conjugation reactions

2.3.1. Before you add the solution of antibody to the Fluorescein label, add 20µl of Fluoro-Trap™ reaction buffer for each 100µl volume of antibody to be labeled. Mix gently.

2.3.2. Remove the screw cap from the vial of Fluorescein and pipette the antibody sample (with added Fluoro-Trap™ reaction buffer) directly onto the lyophilized material. Resuspend *gently* by withdrawing and re-dispensing the liquid a few times using a pipette.

If your reaction volume is in excess of 1.25ml, resuspend the lyophilized material in the volume of reaction buffer given in step 2.3.1 and add the resuspended fluorescein to your sample.

2.3.3. Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25°C).

2.3.4. After incubating for 3 hours, add 10µl of Fluoro-Trap™ Quencher for every 100µl of antibody used.

2.3.5. Incubate with the quencher for 1 hour (a longer period of incubation is okay).

2.3.6. Transfer the reaction mix to the tube insert which contains Fluoro-Trap™ resin. Agitate the contents periodically or place on an end-over-end or other rotary mixing device. The Fluoro-Trap™ resin will turn orange as the free dye is absorbed from the solution of conjugate. This process is complete after 10 minutes.

2.3.6. Centrifuge (up to 4000g) the collecting tube (with its tube insert) for ~20 seconds to collect the purified conjugate in the bottom of the collecting tube. The free dye remains associated with the Fluoro-Trap™ resin in the tube insert.

2.3.7. Discard the tube insert and spent resin and cap the collecting tube.

2.3.8. Add 20µl of neutralizing solution for each 100ul of conjugate.

2.4 Storage of conjugates

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

Appendix 1. Compatibility of buffers and buffer additives.

Amine-free buffers, including MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 7.0-8.5, as the addition of Fluoro-Trap™ reaction buffer provides the conditions necessary for efficient conjugation.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars may be used, as they have no effect on conjugation efficiency.

Avoid buffer components that are nucleophilic, as these may react with the Fluorescein dye. Primary amines (e.g. amino acids, ethanolamine) and thiols (e.g. mercaptoethanol, DTT) fall within this class.

Note: Although Tris is an amine-containing buffer it has little effect on conjugation efficiency as long as the concentration is 50mM or less.

Azide causes significant interference and ideally should be removed or at least reduced in concentration to <0.01%.

Frequently asked questions

Q1. What functional groups do I need on my protein?

Fluoro-Trap™ systems requires amine groups on the biomolecule to be labeled. All antibodies have multiple amine functions.

Q2. Do I have to label 1mg of antibody?

No. The amount does not have to be exactly 1mg but this quantity gives a molar ratio of antibody to dye that leads to good labeling for most antibodies. Varying the amount of antibody using a fixed amount of dye (as provided in the kit) can be used to generate conjugates with a varying average number of dye molecules per antibody. Experimentation in the range 0.5-2mg antibody per tube may be useful in fine tuning the performance of conjugates for specific applications.

For the latest tips and FAQs on Fluoro-Trap™ labeling systems check our website: www.innovabiosciences.com.