FluoroQuest™ Anti-Fading Kit I

Optimized for Slide Imaging

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 20001	Keep in freezer and avoid exposure to light	Fluorescence microscope

Introduction

The photon output of a dye represents the average number of cycles of excitation followed by fluorescence emission that the dye goes through before it is irreversibly photobleached. The average photon output is defined by the ratio of fluorescence quantum efficiency to photobleaching quantum efficiency. When exposed to excitation light, fluorescence intensity of dyes decreases due to their photooxidation or other photoreactions. It is ideal to have the maximal ratio of fluorescence quantum efficiency to photobleaching quantum efficiency. However, very few fluorescent organic dyes can completely resist photobleaching.

Frequently, when a section has been scanned repeatedly under strong excitation light, dyes could lose significant fluorescence signal before visual evaluation or photography can be accomplished. For example, the photobleaching of fluoresceins (such as FITC-labeled antibodies) has become a major problem in fluorescence microscopy. In severe cases (such as phycoprotein-labeled bioconjugates), a fluorescence image with high resolution cannot even be taken due to the extremely high photobleaching rate.

The main purpose of FluoroQuest™ Anti-Fading Kit is to reduce the dye photobleaching rate, giving researchers longer observation time. The kit contains 3 sampler components for you to select according to the types of imaging experiments. They are all premixed and ready-to-use solutions.

Kit Key Features

Convenient: Uniquely formulated ready-to-use solutions that can be applied to a broad spectrum of samples.

Compatible: Proven to be effective for a variety of fluorescence imaging dyes such as fluoresceins (e.g., FITC), rhodamines (e.g., Texas Red®), coumarins (e.g., AMCA and calcein blue) and UV-excitable dyes (e.g., DAPI, Hoechst, Indo-1 and Fura-2), etc.

Kit Components

Components	Amount	Recommendation
Component A	1 mL	Optimized for FITC and other fluorescein-based imaging experiments.
Component B	1 mL	Optimized for multiplexing imaging. In some cases, it enhances initial fluorescence intensity besides its anti-fading effect.
Component C	1 mL	Optimized for multiplexing imaging with minimal phototoxicity.

Protocol

Brief Summary

Prepare Samples (slides or microplate wells) → Add a drop of a component and mount → Examine the specimen under microscope

1. *Thaw the components*: Thaw all the kit components at room temperature, and keep from light.
2. *Apply anti-fading reagent:* Remove any excess liquid from your specimen. Add a small drop of the selected component to the specimen. If the sample is on a slide or tissue culture dish, carefully place a coverslip on

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the drop, avoiding air bubbles. If the sample is on a coverslip, invert the coverslip on a clean glass slide. Remove any excess anti-fading component.

3. *Prepare samples for imaging:* The anti-fading reagents should be incubated for 2 hours to overnight. For long-term storage, seal the coverslip to the slide with nail polish or a plastic sealant. Mounted slides should be stored at 4 ºC in the dark for optimum sample longevity. The fluorescence imaging would remain stable for many weeks. Samples can be imaged immediately after mounting. A typical image is shown in Figure 1.

   Note: These ready-to-use anti-fading reagents can be applied directly on the washed specimen. Although the reagents have been tested with lots of fixed samples, their optimal anti-fading efficiencies strongly depend on the properties of your samples. We suggest that you try more than one component for your imaging samples to get the ideal component. For example, one component may be more compatible with a fluorescent labeled antibody conjugate (or an enzyme substrate or a special mounting specimens that contain lipophilic plasma membrane stains like DiI) than another one.

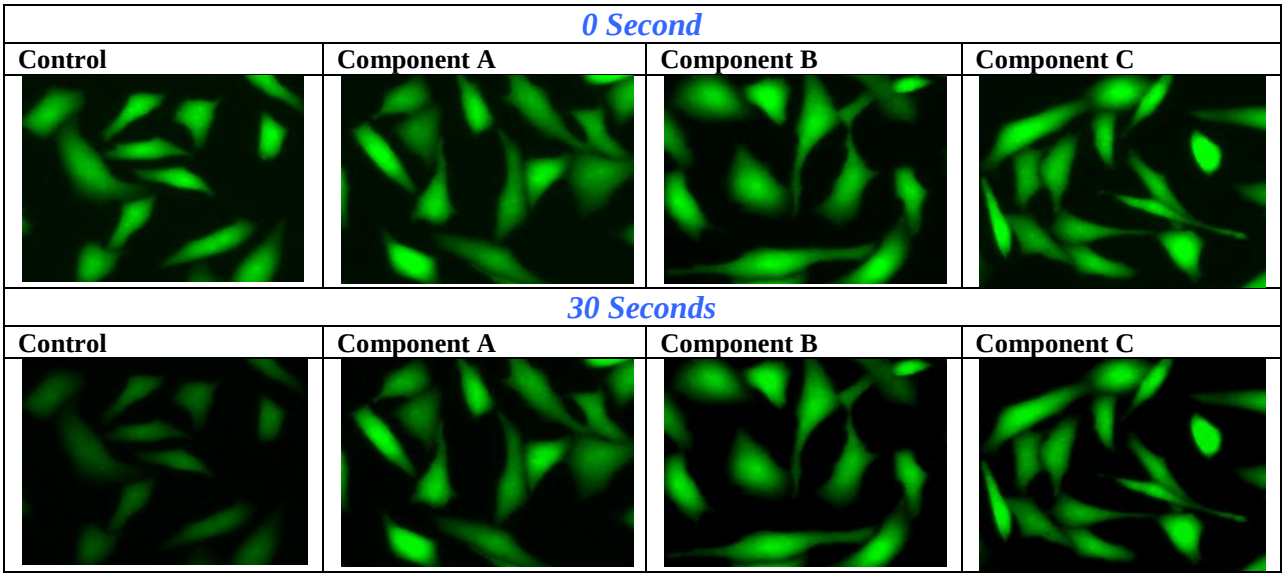

Figure 1. U2OS cells in a 96-well Costar black plate were loaded with 1 µM calcein, AM for 1 hour, fixing with 2% formaldehyde for 30 minutes. Anti-fading reagents were added to the samples after removing all the media. The FITC signals were compared at 0 and 30 seconds exposure time by using an Olympus fluorescence microscopy. The same exposure settings were used for all the images.