FluoroQuest™ Anti-Fading Kit II

Optimized for Plate Imaging

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 20003	Store desiccated at 4 °C and protect from 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 of 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. This kit is formulated for plate images.

Product Key Features

Convenient: Formulated to have minimal hands-on time. It can be applied to a broad spectrum of samples.

Continuous: Easily adapted to automation without a separation step.

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
Component A: Anti-fading Reagent	1 vial
Component B: Assay Buffer	1 vial (10 mL)

Protocol

Brief Summary

Prepare samples (microplate wells) → Remove the liquid from the plate → Add 100 µL/well of anti-fading solution → Examine the specimen under microscope

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Technical Support: support@aatbio.com; Tel: 408-733-1055

1. *Make Anti-fading solution*: Add the entire vial of Assay buffer (Component B) to the vial of Anti-fading Reagent (Component A).
   Note: Aliquot and store the unused anti-fading solution at -20 °C, protected from light. Avoid repeated freeze/thaw cycles. It is still OK to use when the solution goes brown. Discard when the solution is black.

2. *Apply anti-fading reagent:* Remove any excess liquid from your 96-well plate. Add 100 µL of Anti-fading solution (from Step 1) to each selected wells.

3. *Prepare samples for imaging:* Samples can be imaged immediately after apply the Anti-fading solution. A typical image is shown in Figure 1. Store the plate at 4 ºC in the dark for optimum sample longevity.

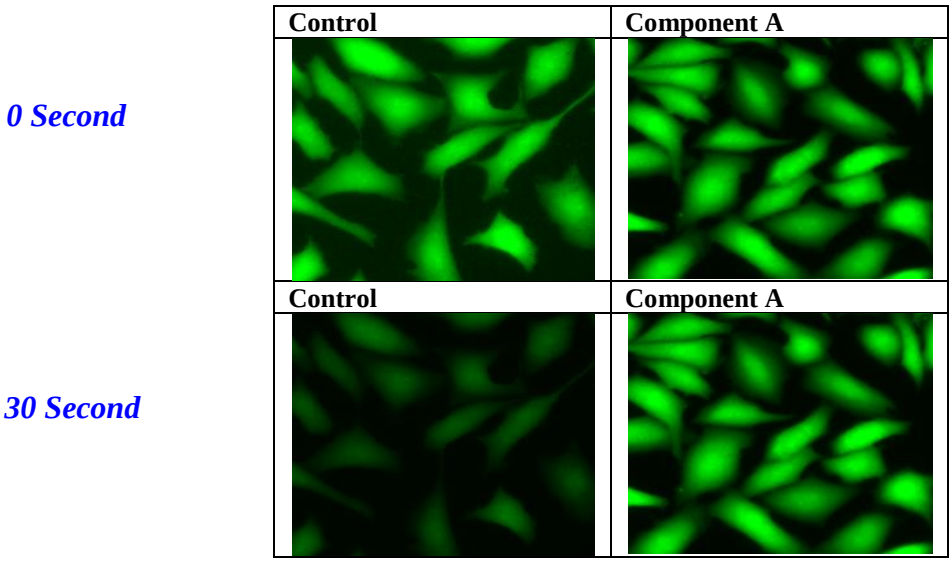

Figure 1. U2OS cells were loaded with 1 µM calcein-AM for 1 hour, fixing with 2% formaldehyde for 30 minutes in a 96-well Costar black plate. Anti-fading reagent (100 µL/well) was added into the samples after removing all the media. The FITC signals were compared at 0 and 30 seconds exposure time by using Olympus fluorescence microscopy. The same exposure settings were used for all the images.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.