

## FluoroQuest™ Anti-fading Kit I \*Optimized for Slide Imaging\*

Catalog number: 20001

Unit size: 1 kit

| Component            | Storage                                   | Amount |
|----------------------|---|--------|
| Component A: AFRG515 | Freeze (<-15 °C), Minimize light exposure | 1 mL   |
| Component B: AFRM520 | Freeze (<-15 °C), Minimize light exposure | 1 mL   |
| Component C: AFRR560 | Freeze (<-15 °C), Minimize light exposure | 1 mL   |

### OVERVIEW

When exposed to excitation light, fluorescence intensity of dyes decreases due to their photooxidation or other photoreactions. There are very few fluorescent dyes that 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 examples, 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 can not even be taken due to the extremely high photobleaching rate. Fluoroquest™ Anti-Fading Kit is to reduce the dye photobleaching rate, giving researchers longer observation time. The kit contains all the essential components that can be readily applied to imaging experiments. They are all premixed and ready-to-use solutions. This kit is designed for slide format while #20003 is designed for microplate format.

### AT A GLANCE

#### Protocol summary

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

**Important** 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.

### KEY PARAMETERS

|                    |                         |
|--------------------|-------------------------|
| Instrument:        | Fluorescence microscope |
| Excitation:        | Varies                  |
| Emission:          | Varies                  |
| Recommended plate: | Black wall/clear bottom |

### PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit <https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

### SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Recommendations for usage

| Components  | Recommendation  |
|-------------|---|
| Component A | Optimized for FITC and other fluorescein-based imaging experiments. |

|             |   |
|-------------|---|
| Component B | Optimized for multiplexing imaging. In some cases, it enhances initial fluorescence intensity besides its anti-fading effect. |
| Component C | Optimized for multiplexing imaging with minimal phototoxicity   |

1. Thaw all the kit components at room temperature, and keep from light.
2. 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 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. 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°C 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.

### EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at: <https://www.aatbio.com/products/fluoroquest-anti-fading-kit-i-optimized-for-slide-imaging>

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