FluoroQuest™ Fluorescence Signal Enhancing Solution

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 20006 (5 mL)	<-15°C, Protect from light.	Fluorescence microscope

Biological Applications

When proteins especially antibodies are conjugated to fluorescent organic dyes, the background fluorescence to unspecific targets might reduce its detection limit significantly due to the decreased affinity caused by fluorophores bound to the proteins. The maximum ratio of fluorescence signal to background is required to obtain the best quality of fluorescentce images for cells. AAT Bioquest's FluoroQuest™ fluorescence signal enhancing solution is a ready-to-use, and provides an effective way to reduce background fluorescence, increase the ratio of fluorescence signal to background for the biological detections using fluorescent conjugates used in immunology, histochemistry, and cell biology.

Storage Conditions

Store at <-15°C. Protect from light. Expiration date is 6 months from the date of receipt.

Typical Assay Protocol for Cell Staining

1. Plate cells on slide chambers or sterile glass coverslips, and treat the cells as desired
2. Perform formaldehyde fixation. Incubate cells with 3.0–4.0 % formaldehyde in PBS at room temperature for 10–30 minutes.
3. Wash the fixed cells 2–3 times in PBS.

   Optional: Add 0.1% Triton X-100 in PBS into fixed cells for 3 to 5 minutes to increase permeability. Rinse the cells 2–3 times in PBS.
4. Block with blocking agent such as with 5% BSA in PBS for 30 min.
5. Dilute primary antibody in dilution buffer as recommended in the specific product's datasheet. Overlay enough diluted antibody to cover cells on coverslip or each chamber of the chamber slides.
6. Wash 2-3 times with PBS.
7. Warm up FluoroQuest™ fluorescence signal enhancing solution at room temperature before use.
8. Dilute fluorescent secondary antibody in FluoroQuest™ fluorescence signal enhancing solution and then stain cells for 1 hour at room temperature. General range for secondary antibodies is between 0.1-10 µg/mL for IgG conjugates for most applications. Keep slips covered or in a humidified chamber to avoid evaporation.
9. Wash the cells three times with PBS.

   Note: Additional staining with fluorescent nuclear stains or phalloidins can be done at this step.
10. Invert each coverslip onto a cleaned slide with mounting media, preferably one with an anti-fade preservative. Seal edges with clear polish if desired.
11. Imaging the cells under microscope with correct filters. Store slides in the dark at 4°C.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

Data Analysis

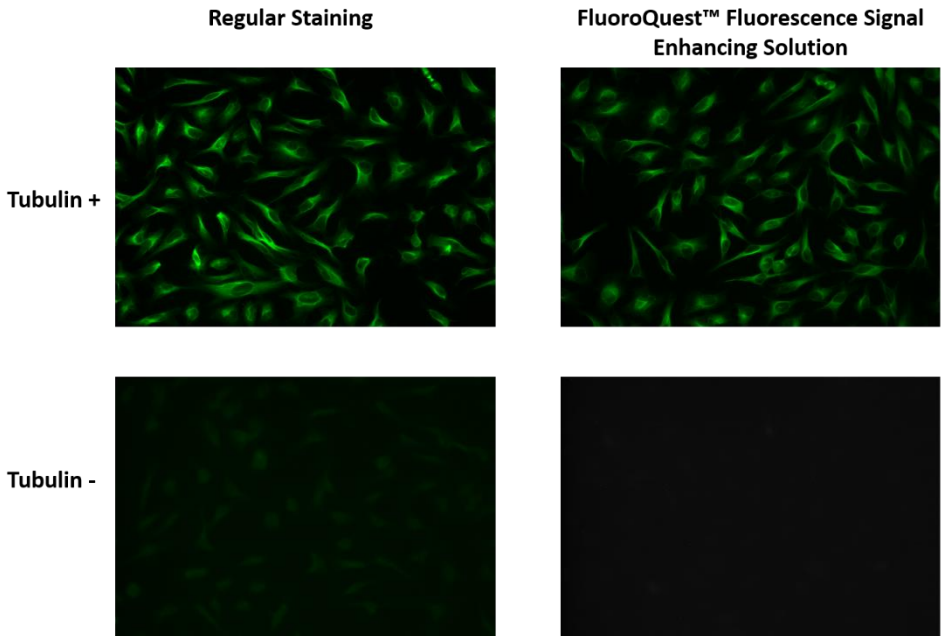

Figure 1. HeLa cells were incubated with (Tubulin+, Upper) or without (Tubulin-, Bottom) mouse tubulin antibody for 30 minutes at room temperature. After 3 times wash in PBS, cells were stained using iFluor™ 488 goat anti-mouse IgG conjugate (Cat#16528) diluted without (Left) or with FluoroQuest™ fluorescence signal enhancing solution (Right), respectively.