Revised: 01–February–2001

Alexa Fluor® 488 Signal-Amplification Kit for Mouse Antibodies (A-11054)

Quick Facts

Storage upon receipt:

- 4°C or –20°C (in aliquots)
- Avoid freeze-thaw cycles
- Protect from light

Number of assays: 60–300

Introduction

The Alexa Fluor® 488 Signal-Amplification Kit for Mouse Antibodies is designed to sensitively detect mouse primary antibodies using immunofluorescence. The kit takes advantage of the superior properties of Alexa Fluor 488 conjugates. Alexa Fluor 488 conjugates are considerably brighter and more photostable than fluorescein-labeled probes. In addition, the fluorescence of Alexa Fluor 488 conjugates is pH-independent over a wide range, unlike the fluorescence of fluorescein conjugates.

To detect antibodies derived from mouse, the Alexa Fluor 488 Signal-Amplification Kit for Mouse Antibodies uses two Alexa Fluor 488 conjugates. Alexa Fluor 488 rabbit anti–mouse IgG, which is prepared from an affinity-purified antibody that has been adsorbed against human serum to minimize crossreactivity, is first used to bind to the mouse primary antibody. The fluorescence signal is then dramatically enhanced by the addition of Alexa Fluor 488 goat anti–rabbit IgG, which is prepared from an affinity-purified antibody that has been adsorbed against human IgG and serum, mouse IgG and serum and bovine serum to minimize crossreactivity. Because the spectra of Alexa Fluor 488 conjugates are remarkably similar to those of fluorescein conjugates (Figure 1), the kit can be used with filters or instrument settings appropriate for fluorescein. The Alexa Fluor 488 Signal-Amplification Kit for Mouse Antibodies can be used for both fluorescence microscopy (Figure 2) and flow cytometry (Figure 3).

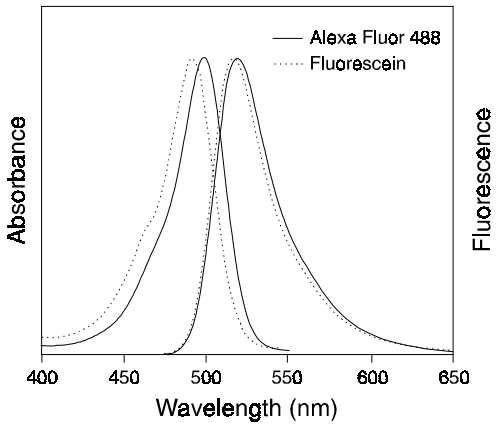

Figure 1. *Normalized absorption and fluorescence emission spectra of Alexa Fluor 488 and fluorescein goat anti–mouse IgG conjugates.*

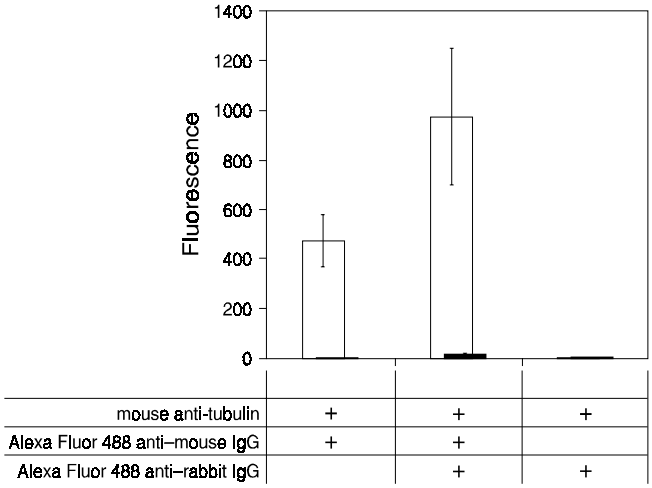

Figure 2. *An example of fluorescence microscopy results obtained using the Alexa Fluor 488 Signal-Amplification Kit for Mouse Antibodies. Bovine pulmonary artery endothelial (BPAE) cells were stained with anti–α-tubulin mouse monoclonal 236-10501 (A-11126) and, as indicated, with Alexa Fluor 488 rabbit anti–mouse IgG (Component A) and Alexa Fluor 488 goat anti–rabbit IgG (Component B). The fluorescence values of the negative controls, in which the anti-α-tubulin antibody was omitted, are shown (black) together with the fluorescence values of the experimental samples (white). Images were acquired using a bandpass filter set appropriate for fluorescein, and fluorescence was quantified using a CCD camera system. Fluorescence values obtained from ten different representative cells were averaged to give the numbers plotted here.*

Materials

Kit Contents

- **Alexa Fluor 488 rabbit anti–mouse IgG** (Component A), 150 µL of a 2 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, 5 mM sodium azide

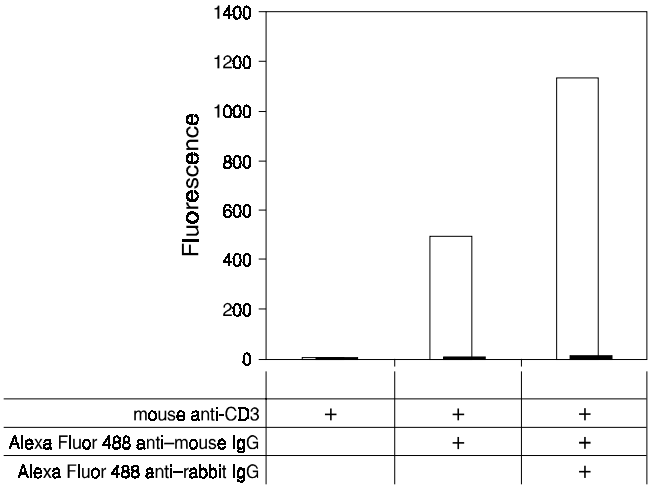

Figure 3. *An example of flow cytometry results obtained using the Alexa Fluor 488 Signal-Amplification Kit for Mouse Antibodies. Human T cell leukemia cells (Jurkat) were stained with mouse anti-CD3 antibody and, as indicated, with Alexa Fluor 488 rabbit anti–mouse IgG (Component A) and Alexa Fluor 488 goat anti–rabbit IgG (Component B). The fluorescence values of the negative controls, in which the anti-CD3 antibody was omitted, are shown (black) together with the fluorescence values of the experimental samples (white). The fluorescence values represent the average signals from the population of cells analyzed.*

- **Alexa Fluor 488 goat anti–rabbit IgG** (Component B), 150 µL of a 2 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, 5 mM sodium azide

Storage and Handling

Upon receipt, the kit components should be stored refrigerated at 4°C, protected from light. When stored properly, the kit components will remain stable for at least three months. For longer storage, divide each component solution into small aliquots and freeze at -20°C, protected from light. Frozen aliquots are stable for at least six months. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Fluorescence Microscopy Protocol

The following protocol is designed to amplify the fluorescence signal of fixed, adherent cells grown on coverslips that have first been stained with a mouse-derived primary antibody. This protocol should be used as an example procedure. Depending on the sample being analyzed, concentrations of the antibody conjugates will need to be adjusted to obtain optimal results. In the protocol, staining solutions are prepared using phosphate-buffered saline (PBS), pH 7.2, containing 1% bovine serum albumin (BSA, RIA grade). With the addition of sodium azide to a final concentration of 5 mM, the BSA–PBS solution can be stored for approximately one month at 4°C. We recommend preparing the individual staining solutions just prior to use.

The volumes recommended here are for staining 5–10 coverslips of adherent cells. Volumes should be adjusted accordingly to stain a different number of samples.

1.1 Fix and permeabilize cells grown on coverslips using an appropriate method (note **A**).

1.2 If desired, treat cells with a blocking agent to reduce nonspecific binding (note **B**).

1.3 Stain the cells with your mouse-derived primary antibody.

1.4 While incubating your samples with the primary antibody, prepare a 10 µg/mL solution of Alexa Fluor 488 rabbit anti–mouse IgG by adding 5 µL of 2 mg/mL Alexa Fluor 488 rabbit anti–mouse IgG (Component A) to 995 µL of PBS containing 1% BSA (BSA–PBS) (note **C**).

1.5 Wash the cells three times with BSA–PBS. Washing can be accomplished by flooding the coverslip with BSA–PBS solution and then quickly inverting the coverslip to decant the solution.

1.6 Drain off the excess BSA–PBS, using a tissue to wick all possible buffer from the coverslips.

1.7 Place the coverslips on a dry surface (e.g., in a dry tissue culture dish or on a piece of Parafilm®). Carefully apply 100–200 µL of the 10 µg/mL Alexa Fluor 488 rabbit anti–mouse IgG solution (prepared in step 1.4) to each coverslip, being sure to completely cover the cells. For standard 18 mm × 18 mm coverslips, we have found that ~100 µL of staining solution works well.

1.8 Cover the samples (e.g., with a tissue culture plate lid) and incubate at room temperature for 30 minutes.

1.9 While the samples are incubating, prepare a 10 µg/mL solution of Alexa Fluor 488 goat anti–rabbit IgG by adding 5 µL of 2 mg/mL Alexa Fluor 488 goat anti–rabbit IgG (Component B) to 995 µL of BSA–PBS (note **C**).

1.10 Wash the cells three times with BSA–PBS, as described in step 1.5.

1.11 Drain off the excess BSA–PBS, using a tissue to wick all possible buffer from the coverslips.

1.12 Place the coverslips on a dry surface (e.g., in a dry tissue culture dish). Carefully apply 100–200 µL of the 10 µg/mL Alexa Fluor 488 goat anti–rabbit IgG solution (prepared in step 1.9) to the samples, being sure to completely cover the cells. For standard 18 mm × 18 mm coverslips, we have found that ~100 µL of staining solution works well.

1.13 Cover the samples (e.g., with a tissue culture plate lid) and incubate at room temperature for 30 minutes.

1.14 Wash the cells twice with BSA–PBS as described in step 1.5.

1.15 Wash the cells once with deionized water.

1.16 Mount the coverslips onto microscope slides in PBS or other suitable mounting medium (note **D**).

1.17 Analyze the samples by fluorescence microscopy using optical filter sets appropriate for fluorescein. The samples will have absorption/emission maxima ~495/519 nm.

Flow Cytometry Protocol

The following protocol is designed to amplify the signals of live cells that have been probed with a mouse-derived primary antibody. The staining protocol has been optimized using a concentration of ~10^6 cells per mL. In the protocol, staining solutions are prepared using phosphate-buffered saline (PBS), pH 7.2, containing 1% bovine serum albumin (BSA), and all staining and wash steps are performed on ice. With the addition of sodium azide to a final concentration of 5 mM, the BSA–PBS solution can be stored for approximately one month at 4°C.

2.1 Harvest the cells by an appropriate method (e.g., trypsinization followed by gentle centrifugation) and transfer the cells to test tubes or centrifuge tubes. Wash the cells in ice cold BSA–PBS and then add sufficient BSA–PBS to resuspend the cells at an appropriate concentration for your staining protocol (notes **E**, **F**).

2.2 Stain the cells with your mouse-derived primary antibody at the appropriate concentration.

2.3 Wash the cells twice with ice cold BSA–PBS (note **E**). Resuspend the cells in BSA–PBS at ~10^6 cells per mL.

2.4 Add the appropriate amount of 2 mg/mL Alexa Fluor 488 rabbit anti–mouse IgG (Component A) to obtain 5 µg of conjugate per million cells. For example, to 1 mL of cells at ~10^6 cells/mL, add 2.5 µL of 2 mg/mL Alexa Fluor 488 rabbit anti–mouse IgG (note **G**). Mix well.

2.5 Incubate for 30 minutes on ice.

2.6 Wash the cells twice with BSA–PBS (note **E**).

2.7 Add the appropriate amount of Alexa Fluor 488 goat anti–rabbit IgG (Component B) to obtain 5 µg of conjugate per million cells. For example, to 1 mL of cells at ~10^6 cells/mL, add 2.5 µL of 2 mg/mL Alexa Fluor 488 goat anti–rabbit IgG (note **G**). Mix well.

2.8 Incubate the samples on ice for 30 minutes.

2.9 Wash the cells two times with BSA–PBS (note **E**). Resuspend the cells in PBS to achieve the desired number of cells per mL (note **H**).

2.10 Analyze the cells by flow cytometry, using settings appropriate for fluorescein-labeled cells. The samples will have absorption/emission maxima ~495/519 nm.

Notes

[A] We have found the following fixation protocol to work well. Wash the cells in PBS and then treat with 3.7% formaldehyde for 30 minutes at 37°C. Wash three times in PBS. Cells can be permeabilized by adding 0.2% Triton® X-100 in PBS to the fixed cells and incubating for 5 minutes at room temperature. Wash cells three times in PBS after permeabilization.

[B] Cells can be blocked by treating with PBS containing 6% BSA for 30 minutes at room temperature. Wash three times in PBS prior to staining.

[C] For optimization of the protocol, we suggest trying concentrations of 5–15 µg/mL for each antibody conjugate and observing the staining after each labeling step.

[D] Cells can be mounted in PBS by placing the coverslip cell-side down on top of a drop (e.g., 10 µL) of PBS. The coverslip should then be sealed with paraffin or other sealing agent. Alternatively, the samples can be mounted in a permanent mounting medium if long-term storage is desired. We have found that the ProLong® Antifade Kit (P-7481), available from Molecular Probes, works well for this purpose.

[E] Washing can be accomplished by filling the tubes with BSA–PBS and then gently pelleting the cells by centrifugation. The supernatant can be easily removed by aspiration.

[F] For example, we have found that ~5×10^6 cells/mL is an appropriate dilution for many staining protocols.

[G] For optimization of the protocol, we suggest trying concentrations of 5–15 µg of conjugate per million cells (at ~10^6 cells per mL) for each antibody conjugate. Because the fluorescence intensity may be sufficient after staining with only the Alexa Fluor 488 rabbit anti–mouse IgG conjugate (Component A), you may wish to analyze samples after the first staining step and again after labeling with the Alexa Fluor 488 goat anti–rabbit IgG (Component B). Please note that quenching of the fluorescence signal may occur if targets are overlabeled.

[H] If desired, propidium iodide can be added to discriminate dead cells from live cells. We have found that addition of 1 µL of 1 mg/mL propidium iodide to each sample (~10^6 cells) is sufficient for dead cell discrimination.

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A-11054	Alexa Fluor® 488 Signal-Amplification Kit for Mouse Antibodies *60-300 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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