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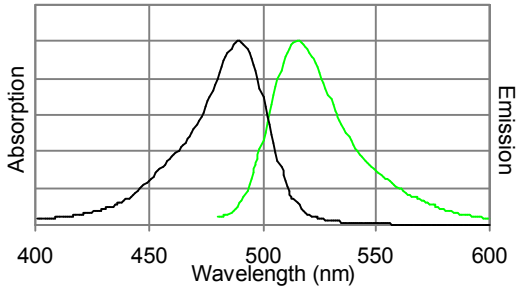
Product Information

Antibody–CF™488A Conjugates
affinity isolated antibody

Product Description

Antibody–CF™488A conjugates are affinity-purified antibodies labeled with a green fluorescent dye, CF488A. CF dyes are excellent alternatives to Alexa Fluor® dyes for antibody labeling with exceptional brightness, photostability, and/or specificity. CF488A is spectrally similar to Alexa Fluor 488, Cy™2, DyLight®488, FAM, and fluorescein (FITC) (see Figure 1).

Figure 1.
Absorption/Emission Spectra of CF488A Conjugated Antibodies



λ_{abs} = 490 nm (PBS buffer, pH 7.4)
λ_{em} = 515 nm (PBS buffer, pH 7.4)

Reagent

Supplied as a solution in PBS, pH ~7.4, containing 50% glycerol, 2 mg/ml bovine serum albumin (IgG-free and protease-free), and 0.05% sodium azide as a preservative.

Antibody Concentration: 2 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Product remains active for about 6 months at –20 °C as an undiluted liquid. Protect from light. Storage of the antibody for more than a day at final working dilution is not recommended.

Product Profile

The suggested dilution range for antibody–CF488A conjugates for immunofluorescence and flow cytometry applications is 1–10 µg/mL.

Antibody–CF488A conjugates also can be used for staining histological sections from paraffin-embedded or frozen tissues.

Note: In order to obtain the best results using various techniques and preparations, it is recommended the end user determine the optimal working dilution for their system by titration assay.

Procedures

A. Immunofluorescence

There are many methods for immunofluorescence staining. This protocol is a general guideline for staining cells, and should be optimized or modified to obtain the best results for each particular application.

Coverslip preparation for adherent cells

1. Culture cells on slide chambers or sterile glass coverslips. 18 × 18 mm square coverslips in 6 well plates or 4 well chamber slides are recommended. If cells do not adhere well to glass, use Coverslip preparation for non-adherent cells (poly-L-lysine coated coverslips).
2. Allow cells to adhere and treat as desired.
3. Rinse cells gently with PBS.

Coverslip preparation for non-adherent cells

1. Coat coverslips with 0.01% poly-L-lysine solution for 10 minutes at room temperature.
2. Aspirate the poly-L-lysine solution and allow coverslips to dry completely.
3. Centrifuge cells in medium and resuspend in PBS. Transfer cells to coverslips.
4. Incubate for 30–60 minutes. Check for adherence by microscopy.

Fixation and Staining

1. Fix with 4% paraformaldehyde/PBS solution for 15 minutes.
2. Rinse twice with PBS to remove traces of fixative.
3. Permeabilize with 0.1–0.5% Triton™ X-100/PBS for 5–10 minutes.
4. Block with blocking buffer (5% BSA or normal donkey serum in PBS) for 30 minutes.
5. Dilute primary antibody as recommended by manufacturer's datasheet. Overlay enough diluted antibody to cover cells on coverslip (150–200 μL is usually sufficient to cover the surface area) or add to each chamber of the chamber slides. Keep slips covered or in a humidified chamber to avoid evaporation.
6. Rinse three times with PBS for 5 minutes each time.
7. Dilute fluorescent secondary antibody in diluent and incubate for 1 hour at room temperature. General range for IgG conjugates is between 1–10 μg/mL for most applications. Cell samples without primary antibody incubation are recommended for background control. Keep slips covered or in a humidified chamber to avoid evaporation.
8. Rinse three times with PBS for 5 minutes each time.
9. Additional staining with fluorescent nuclear stains or phalloidins can be done at this step.
10. Invert each coverslip onto a precleaned slide with a mounting medium, preferably one with an anti-fade preservative. Seal edges with clear polish if desired.
11. Store slides in the dark at 2–8 °C.

B. Flow Cytometry

There are many alternative procedures that can be used for specific staining experiments. This protocol is a general guideline for flow cytometry and should be optimized or modified for each application.

1. Aliquot 1×10^6 cells into 12×75 mm polypropylene tubes for flow cytometry.
2. For intracellular staining, cells can be fixed first to ensure stability of soluble antigens or antigens with short half-lives. A fix and perm kit from a reliable manufacturer is recommended. Follow manufacturer's instructions.
3. Add the primary antibody or isotype control at the appropriate dilution to the assay tubes. Incubate according to manufacturer's instructions.
4. Rinse cells twice by centrifugation with 2–3 mL of incubation buffer.
5. Decant supernatant and resuspend the pellet in remaining volume of wash buffer.
6. Add fluorescent secondary antibody and incubate for 20–30 minutes. General range for secondary antibodies is between 1–10 μg/mL for IgG conjugates for most applications.
7. Rinse cells twice by centrifugation with 2–3 mL of incubation buffer. Centrifuge to collect cells after each wash. Decant supernatant.
8. Resuspend cells in 0.5 mL of diluent of choice to analyze on flow cytometer. Acquire data using the correct channel.

Troubleshooting

1. No signal or weak fluorescence intensity may suggest the following:
   - insufficient antibody is present for detection
   - intracellular target was not accessible
   - excitation sources are not aligned
   - target protein is not present or expressed at low levels
   - fluorochrome has faded, and/or
   - primary and secondary antibodies are not compatible.

2. High fluorescence intensity may suggest the following:
   - antibody concentration is too high
   - excess antibody was not washed away efficiently, and/or
   - blocking was inadequate.

   Increase antibody dilution and washes.

References

1. Donaldson, J.G., Immunofluorescence staining, Curr. Protoc. Cell Biol., Chapter 4, Unit 4.3 (2001).
2. Blose, S.H., and Feramisco, J.R., Fluorescent methods in the analysis of cell structure. Cold Spring Harbor Laboratory Press (1983).

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