INSTRUCTIONS

MIST Bioscience

MIST Linker™ Cleavable Antibody-Fluorophore Labeling Kit for Mouse IgG2a/b

REF: **MCFL-488M2-10; MCFL-543M2-10; MCFL-647M2-10; MCFL-750M2-10**
MCFL-488M2-20; MCFL-543M2-20; MCFL-647M2-20; MCFL-750M2-20
MCFL-488M2-50; MCFL-543M2-50; MCFL-647M2-50; MCFL-750M2-50

Description:

MIST Linker cleavable labeling kit for labeling mouse IgG2a/b antibodies with UV cleavable fluorophores

Target: Primary antibodies with isotype mouse IgG2a/b. Compatible with most antibody storage buffers.

Note: Each kit supports 10 labelings (MCFL-488M2-10; MCFL-543M2-10; MCFL-647M2-10; MCFL-750M2-10), 20 labelings (MCFL-488M2-20; MCFL-543M2-20; MCFL-647M2-20; MCFL-750M2-20), or 50 labelings (MCFL-488M2-50; MCFL-543M2-50; MCFL-647M2-50; MCFL-750M2-50) of monoclonal or polyclonal mouse IgGa/b antibodies with fluorophores. The product is recommended to process **0.5 µg** antibody per test, depending on antibody concentration.

If cleavage of fluorophore is needed, a UV light source such as a typical laboratory handheld UV lamp (365 nm, 3–10 W) is required.

Storage: Upon receipt the kit should be stored at 4 °C in the dark. The product may be shipped at ambient temperature.

For Research Use Only. Not for use in diagnostic procedures.

Introduction

The MIST Bioscience MIST Linker Antibody-Fluorophore Labeling Kit enables one-step, rapid site-specific labeling of minimal amounts of antibody from various sources with fluorophores. Upon UV exposure, the fluorophores on labeled antibodies will typically be cleaved with >90% efficiency under recommended conditions. Each kit includes a MIST Linker reagent labeled with a type of fluorophore, dilution buffer, and MIST Quencher™ resin. The MIST Linker molecule binds only to the Fc region of mouse IgG2a/b antibodies with minimal interference with Fab binding affinity. An antibody of 0.5 µg can be labeled with fluorophore in as little as 5 minutes. The kit also includes MIST Quencher resin to remove excess reactive MIST Linker molecules. With appropriate use, typically >80% recovery is achievable with only 0.5 µg input antibody.

A. Labeling primary antibodies with fluorophores via MIST Linker

1. Calculate the amount of antibody and the required MIST Linker reagent. **1 µL MIST Linker can label 0.5 µg antibody**.

Note: Estimate the antibody concentration from the vendor's information if it is not provided. The majority of commercial antibodies are supplied at around 0.1–1 mg/mL. Over-labeling with excess MIST Linker may not cause obvious adverse effects since the excess MIST Linker is removed in the purification step. If the antibody solution contains 50% glycerol, dilute it 5x with dilution buffer before mixing with MIST Linker.

!Important: MIST Linker should always be kept in the dark. Minimize exposure to ambient light.

2. Pipette and mix antibody with the appropriate amount of MIST Linker in a microcentrifuge tube, briefly centrifuge down, and incubate at room temperature for 5 minutes in the dark.

Note: Longer incubation time may be beneficial for the labeling volume over 5 µL. If the volume of MIST Linker and antibody mixture is too small and not visible, add 1-5 µL dilution buffer before incubation. But ensure the total volume does not exceed 10 µL.

3. Vortex the MIST Quencher bead stock thoroughly to ensure a uniform suspension. Aliquot 5 µL of the bead suspension into the antibody and MIST Linker mixture. Incubate the solution on an orbital shaker for 5 - 10 minutes at room temperature, or manually agitate (mix by inversion) periodically to maintain homogeneity.

4. Briefly spin down for 5 seconds or keep the tube upright for 30 seconds so the resin settle to the bottom.

5. Carefully aspirate the supernatant without taking up any resin in the pellet. The collected liquid contains purified antibody-fluorophore conjugate. Dilute it in an appropriate buffer and with appropriate dilution times for downstream experiments. The undiluted conjugate can be kept in a 4 °C refrigerator for up to 1 week. Longer preservation is possible but not guaranteed.

Note: In principle, the whole process does not lose antibody. The yield is dependent on the ratio between the supernatant and the MIST Quencher bead pellet, which is less than 2 µL. To recover more labeled antibody, optionally add an additional 10 µL of dilution buffer or a buffer compatible with downstream experiments before centrifugation.

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B. Example protocol of immunofluorescence staining with labeled antibodies:

The following protocols are provided strictly as illustrative examples of how users may apply their finalized, purified antibody-fluorophore conjugates in downstream staining applications. These workflows are independent of the MIST Linker™ kit operations.

B.1 Immunofluorescence staining of cultured cells

1. Preparation of cell culture medium: Mix 890 mL of RPMI medium with 100 mL of fetal bovine serum (FBS) and 10 mL of penicillin-streptomycin. Mix thoroughly, aliquot as needed, and store at 4 °C until use.

2. Seed the cells into a 96-well plate and incubate at 37 °C with 5% CO_2 in an incubator.

3. When cells have fully adhered to the surface and reach an ideal confluency, stop the culture. Wash the cells three times with phosphate-buffered saline (PBS; pH 7.2-7.4), then fix them with 4% formaldehyde solution (prepared in PBS) at RT on a shaker at 30 rpm for 10 minutes. After fixation, wash the cells three times with PBS (pH 7.4).

4. Permeabilize cells with 0.1% Triton X-100 at RT for 10 minutes on a shaker at 30 rpm. After permeabilization, wash the cells three times with PBS.

5. Block the cells using the blocking buffer at RT on a shaker at 30 rpm for 1 hour.

6. After removing the blocking buffer, dilute the labeled antibody (from **Section A**) into an antibody staining buffer (e.g., 1% BSA in PBS). The total volume of dilution depends on the affinity of antibodies. Add the labeled antibody solution to the wells of the 96-well plate and incubate with the cells at RT on a shaker at 30 rpm for 1–3 hours or overnight at 4 °C.

Note: For multiplex IF, simply mix the labeled antibodies (from **Section A**) of different fluorophores and dilute the mixture appropriately.

7. Following the incubation step, wash the cells three times with PBS solution. Subsequently, add an appropriate volume of PBS solution to each well of the 96-well plate, and image the cells under a fluorescence microscope equipped with appropriate filter sets.

8. (*optional*) To label other antibodies using the same sample, expose the wells to UV light for 10 minutes. Observe the remaining fluorescence under the microscope to ensure sufficient cleavage. Depending on the sample source and experimental requirement, additional fluorescence suppression methods may be needed.

Caution: UV light can injure the eyes and skin. Wear UV-blocking eyewear and avoid direct skin and eye exposure during photocleavage.

Note:

(1) Cells can be pre-treated and blocked in parallel with the preparation of the antibody conjugate (**Section A** and **B**).

(2) The staining (Step 6) can use higher concentration of labeled antibody, 37 °C incubation and longer incubation time for the detection of low-abundance proteins, or when high sensitivity is required. Overnight incubation at 4 °C may improve staining but is not generally needed.

(3) It is critical to avoid cell drying at any time to minimize high background signal.

B.2 Immunostaining of FFPE tissue sections

1. Bake paraffin-embedded tissue sections at 70 °C for 30 minutes.

2. Deparaffinization: Prepare fresh solutions in 50 mL centrifuge tubes for the following washes:

Solution	Time	Repeats
100% Xylene	3 minutes	2 times
Xylene : 100% EtOH (1:1)	3 minutes	1 time
100% EtOH	3 minutes	1 time
95% EtOH	3 minutes	1 time
70% EtOH	3 minutes	1 time
50% EtOH	3 minutes	1 time
DI water	3 minutes	2 times

For each wash, fully submerge the slides in the solution contained within a 50 mL centrifuge tube. Gently move the slides within the solution during each wash step to promote thorough deparaffinization and hydration. After processing, store the sections in a PBS solution at 4 °C for future use.

Note:

(1) Always perform xylene and xylene-ethanol steps inside a chemical hood.

(2) Fresh xylene and ethanol solutions are recommended for best results.

(3) Handle slides carefully to prevent tissue detachment during washes.

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3. Permeabilize tissue sections with 0.3% Triton X-100 at RT for 10 minutes on a shaker at 30 rpm. After permeabilization, wash the samples three times with PBS.

4. Block the tissue sections using a tissue blocking buffer (e.g., 5% goat serum in PBS), incubating at RT on a shaker at 30 rpm for 1 hour.

5. After removing the blocking buffer, dilute the labeled antibody (from **Section A**) in an antibody staining buffer (e.g., 1% BSA in PBS). The total volume of dilution depends on the affinity of antibodies. Add the labeled antibody solution to the sections and incubate at RT on a shaker at 30 rpm for 1-3 hours or overnight at 4 °C.

Note: For multiplex IF, simply mix the labeled antibodies (from **Section A**) of different fluorophores and dilute the mixture appropriately.

6. Following the incubation step, wash the tissues three times with PBS. Subsequently, add an appropriate amount of mounting buffer to the sample and cover it with a coverslip for fluorescence microscopy imaging.

7. (*optional*) To label other antibodies using the same section, expose the labeled tissue section to UV light for 10 minutes. Observe the remaining fluorescence under the microscope to ensure sufficient cleavage. Depending on the sample source and experimental requirement, additional fluorescence suppression methods may be needed.
Caution: UV light can injure the eyes and skin. Wear UV-blocking eyewear and avoid direct skin and eye exposure during photocleavage.

Note:

(1) Tissues can be pre-treated and blocked in parallel with the preparation of the conjugate (**Section A**).

(2) The staining (Step 6) can use higher concentration of labeled antibody, 37 °C incubation and longer incubation time for the detection of low-abundance proteins, or when high sensitivity is required. Overnight incubation may improve labeling efficiency.

(3) It is critical to avoid tissue drying at any time to minimize high background signal.

IMPORTANT NOTICE: THIRD-PARTY REAGENTS & SAFETY

MIST Bioscience does not provide or warrant the third-party reagents, materials, or equipment listed in Section B (e.g., cell culture media, fixatives, permeabilization agents, solvents, or mounting media). Users assume full responsibility for the safe handling, storage, and disposal of all chemicals used in downstream assays**.** All procedures should be performed in accordance with your institution's Standard Operating Procedures (SOPs) and the respective chemical manufacturers' Safety Data Sheets (SDSs).

Troubleshooting

Problem	Possible Cause	Solution
High background	Non-specific binding	(1) Always keep tissues/cells from drying during all steps. During cell staining, keep the lid on the 96-well plate. During tissue staining, place the tissue in a humidified dish; (2) Wash samples more times, and completely remove all solutions between washing steps; (3) Increase blocking time; (4) Optimize labeling concentration.
	Excess MIST Linker not sufficiently removed, or improper use of MIST Quencher resin	(1) Follow the protocol. Or, purify the conjugate again by MIST Quencher resin. (2) Apply the correct MIST Quencher specific to the antibody.
	MIST Linker residue stained on tissue	(1) Follow the protocol using MIST Quencher resin; (2) Use 1:200 diluted MIST Linker to stain the sample to test if there is nonspecific binding.
	Endogenous autofluorescence interference	Suppress autofluorescence before staining.
Bright punctate spots in background	Conjugate aggregation	Use freshly prepared antibody-fluorophore conjugates.
	Washing is not effective	(1) Wash the samples with longer duration and more times; (2) Use PBST or blocking buffer to wash.
Weak signal	Poor labeling quality	(1) Check if the antibody host isotype matches the right MIST Linker kit; (2) Increase MIST Linker amount; (3) Purify antibody before labeling.

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	Loss of conjugates in purification	(1) Add dilution buffer or a buffer compatible with downstream experiments before centrifugation of MIST Quencher resin; (2) Ensure antibody does not have any tag.
	Incompatible mounting medium	Change antifade mounting medium types.
	Photobleaching or cleavage	Minimize light exposure from ambient light or bright field light during preparation and imaging. Avoid DAPI channel imaging before other channels.
	The target proteins have low abundances in samples	(1) Increase incubation time of labeled antibody conjugates; (2) Concentrate the antibody to reach about 0.5 mg/mL.
Strong autofluorescence	Autofluorescence from tissue specific regions	(1) Use additional quenching treatments before immunofluorescence staining; (2) Use longer-wavelength dyes.
	Autofluorescence from experimental process	(1) Manually scrape thick paraffin surrounding the tissue prior to the deparaffinization process (2) Ensure that hydrophobic pen markings do not detach from the surface.
Strong signal after photocleavage	Insufficient UV exposure	(1) Ensure sufficient UV light penetrates the sample; (2) Use mild antibody stripping solution on the tissue during UV exposure; (3) Apply strong fluorescence quenching solution.
Tissue/cell detachment during protocol	Overly harsh washing or direct contact with samples by pipette tips	Avoid flushing or physically contacting sample surface in any pipetting step. Wash gently.
	Weak tissue/cell adhesion	Use biochemically treated surface for better attachment.

Technical Support

For technical inquiries, please get in touch with our technical support team at contact@mistbioscience.com.

Disclaimer

This reagent is intended exclusively for research use and must not be used in diagnostic procedures, clinical applications, therapeutic interventions, or in any form of administration to humans or animals unless explicitly stated and supported by applicable regulatory clearance. All reagents are supplied with the expectation that they will be handled and applied only by trained professionals familiar with laboratory practices and applicable safety protocols. The product is warranted to conform to the specifications outlined in the accompanying product documentation at the time of shipment, provided it is stored, handled, and used strictly according to the recommended guidelines. Performance values stated in these instructions (e.g., cleavage efficiency, recovery, and labeling time) are representative results obtained under MIST Bioscience's internal testing conditions and are provided for guidance only; they do not constitute warranted specifications. Actual results depend on factors outside the seller's control, including antibody quality, reagents, sample type, equipment, and user technique. This limited warranty is valid for a period of three (3) months from the date of shipment and applies solely to the original purchaser. However, due to the sensitive and perishable nature of biotechnology reagents, ALL SALES ARE FINAL, AND PRODUCTS ARE STRICTLY NON-RETURNABLE AND NON-REFUNDABLE, EXCEPT IN CASES WHERE A MANUFACTURING DEFECT IS CLEARLY DEMONSTRATED AND REPORTED IN WRITING WITHIN TEN (10) BUSINESS DAYS OF RECEIPT. The buyer assumes full responsibility for proper storage, handling, and application, and agrees that the seller shall not be held liable for damages or performance failures resulting from improper use, modification, accident, negligence, or use beyond the intended research scope. Except as explicitly provided above, the seller makes no other warranties, express or implied, including but not limited to any implied warranties of merchantability, fitness for a particular purpose, or non-infringement, all of which are hereby disclaimed to the fullest extent permitted by law. Under no circumstances shall the seller be liable for any indirect, incidental, special, punitive, or consequential damages, including loss of data, research interruption, or third-party claims, arising out of or related to the use, misuse, or inability to use this reagent. Use of this product constitutes the buyer's acceptance of these terms and acknowledgment of full responsibility for compliance with all applicable laws and regulations.

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