

MIST Linker™ Cleavable Antibody-DNA Conjugation Kit

REF: MADC-MIgG1-10; MADC-MIgG1-20; MADC-MIgG1-50;
MADC-MIgG2-10; MADC-MIgG2-20; MADC-MIgG2-50;
MADC-RIgG-10; MADC-RIgG-20; MADC-RIgG-50.

Description:

MIST Linker™ cleavable antibody-DNA conjugation kit for linking IgG antibodies to oligonucleotides

Target: Primary antibodies with isotype mouse IgG1 (MADC-MIgG1-10; MADC-MIgG1-20; MADC-MIgG1-50), primary antibodies with isotype mouse IgG2a/b (MADC-MIgG2-10; MADC-MIgG2-20; MADC-MIgG2-50), and primary antibodies with isotype rabbit IgG (MADC-RIgG-10; MADC-RIgG-20; MADC-RIgG-50). All of them are specific to Fc region of those IgGs. Compatible with most antibody sources and storage buffers.

Note: Each kit supports 10 conjugations (MADC-MIgG1-10; MADC-MIgG2-10; MADC-RIgG-10), 20 conjugations (MADC-MIgG1-20; MADC-MIgG2-20; MADC-RIgG-20), or 50 conjugations (MADC-RIgG-50; MADC-RIgG-50; MADC-RIgG-50;) of monoclonal or polyclonal mouse IgG1, mouse IgG2a/b or rabbit IgG antibodies with oligonucleotides. Each conjugation is optimized for **1 µg** of antibody, depending on antibody concentration. Each kit also includes Amicon® 100 kDa ultra centrifugal filters for removing excess oligonucleotides, supplied at three (3) filters per 10 conjugations (3, 6, and 15 filters for the 10, 20 and 50 conjugation kits, respectively). Additional filters may be purchased directly from Sigma-Aldrich; these are third-party products and are not supplied or warranted by MIST Bioscience.

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 cleavable antibody-DNA conjugation (ADC) kit enables two-step, rapid site-specific labeling of minimal amounts of antibody from various sources with oligonucleotide DNAs. Upon UV exposure, the oligonucleotides on the antibodies will typically be cleaved with >90% efficiency under recommended conditions. Each kit includes a MIST Linker ADC reagent, dilution buffer, MIST Quencher™ resin, and Amicon® 100 kDa ultra centrifugal filters. The MIST Linker molecule binds selectively to the Fc region of mouse IgG1, mouse IgG2 or rabbit IgG antibodies, with minimal interference with Fab binding affinity. As little as 1 µg of antibody can be labeled in approximately 1 hour. The MIST Linker is compatible only with biotinylated oligonucleotides (single- or double-stranded DNA, and possibly biotinylated RNA). These are supplied by the user and are not included in the kit. The kit also includes MIST Quencher resin to remove excess reactive MIST Linker, and ultra centrifugal filters to remove excess oligonucleotides. With appropriate handling, recovery typically exceeds 70% from a 1 µg input.

Protocol: Conjugating primary antibodies with oligonucleotide DNAs via MIST Linker

1. Calculate the amount of antibody and the required MIST Linker reagent. **1.5 µL of MIST Linker fully labels 1 µg of antibody.**

Note: A minimum of 1 µg of antibody is required for each conjugation. If the concentration is not provided, estimate it from the vendor's specifications; most commercial antibodies are supplied at 0.1–1 mg/mL. Over-labeling with excess MIST Linker is generally well tolerated, as the excess is removed during the purification step. If the antibody is stored in 50% glycerol, dilute it 5-fold with dilution buffer before adding MIST Linker.

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

2. In a microcentrifuge tube, combine the antibody with the appropriate volume of MIST Linker. Mix, briefly centrifuge, and incubate at room temperature for 5 minutes in the dark.

Note: A longer incubation may be beneficial for conjugation volumes greater than 10 µL. If the combined volume of MIST Linker and antibody is too small to handle reliably, add 1–5 µL of dilution buffer before incubation, ensuring the total volume does not exceed 10 µL.

3. Vortex the MIST Quencher resin suspension thoroughly to ensure uniformity. Add 5 µL of the bead suspension to the reaction. Incubate on an orbital shaker for 5–10 minutes at room temperature, or mix periodically by inversion to keep the resin suspended.

4. Briefly centrifuge (~5 seconds), or stand the tube upright for ~30 seconds, to allow the resin to settle.
5. Carefully aspirate the supernatant without disturbing the bead pellet. The supernatant contains the purified antibody-MIST Linker conjugate.
6. Add 3 μ L biotinylated oligonucleotide DNA at 50 μ M, or an equivalent amount, to the antibody-MIST Linker conjugate. Incubate at room temperature for 30 minutes.

Note: Biotinylated oligonucleotides are not supplied with the kit; obtain them from your laboratory or a commercial vendor. For efficient removal of excess oligonucleotide by the 100 kDa filter in the next step, ssDNA/ssRNA oligonucleotides should ideally be no more than 40–50 nt.

7. (Optional) Dilute the mixture to approximately 400–500 μ L with the provided dilution buffer and transfer it to an Amicon® 100 kDa ultrafiltration filter to remove excess oligonucleotides. Following the filter manufacturer's instructions, concentrate the solution to 30–50 μ L.

Note: Centrifugation speed and time may vary by device; for example, 5,000 rpm for 5 minutes in an Eppendorf 5415D centrifuge is typically sufficient. Avoid over-centrifugation, which can reduce conjugate recovery.

8. (Optional) Recover the retained conjugate from the filter and dilute it in a buffer appropriate to the downstream application. The undiluted conjugate may be stored at 4 °C for up to 1 week; longer storage may be possible but is not guaranteed.

Note: For multiplex assays, complete removal of excess oligonucleotide is critical. When preparing multiple conjugates, combine the products of step 6 and purify them together in steps 7 and 8. The purified conjugate mixture should not be stored and should be used on the same day of purification.

IMPORTANT NOTICE: THIRD-PARTY MATERIALS & SAFETY

Certain reagents and equipment used in this protocol are supplied by the user and are not provided or warranted by MIST Bioscience - in particular, the biotinylated oligonucleotides and any UV light source, centrifuge, or shaker. Third-party manufactured components included in the kit (e.g., Amicon® ultra centrifugal filters) are not warranted by MIST Bioscience beyond the original manufacturer's terms. Users assume full responsibility for the safe handling, storage, and disposal of all materials used in the conjugation workflow and in downstream assays, in accordance with their institution's Standard Operating Procedures (SOPs) and the relevant manufacturers' Safety Data Sheets (SDSs). When using a UV light source, wear appropriate eye and skin protection.

Troubleshooting

Problem	Possible Cause	Solution
Low yield of conjugation	Unexpected cleavage	(1) Keep the entire conjugation process in the dark or under yellow light, and avoid exposure to strong light; (2) Use fresh MIST Linker reagent, stored protected from light.
	Excess MIST Linker not sufficiently removed, or improper use of MIST Quencher resin	(1) Follow the protocol, or repeat the purification with MIST Quencher resin; (2) Add more biotinylated oligonucleotides.
	Unsuitable ultrafiltration filter	Use Amicon® 100 kDa filter. Significant antibody loss has been observed with PES-based filters from other brands.
	Inaccurate antibody quantitation	Re-measure the antibody concentration more accurately.
Insufficient cleavage	Inadequate UV exposure	Increase UV power and/or exposure time.
	Hydrolysis of chemical linker	(1) Use freshly prepared conjugates. (2) Store reagents correctly and protected from light.
Low purity	Excess MIST Linker or oligonucleotides not removed sufficiently	(1) Repeat the MIST Quencher purification to remove excess MIST Linker; (2) Use the 100 kDa filter as directed.
	Oligonucleotide too long	Alternatively, use streptavidin-coated beads to remove excess biotinylated oligonucleotides.
	Incorrect MIST Quencher	Use the MIST Quencher specified for your antibody.

Technical Support

For technical inquiries, please contact 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.