Protocol for Biomolecule Labeling Using ADIBO Dyes

Description

ADIBO reagents are bioorthogonal labeling tools designed for SPAAC (strain-promoted azide-alkyne cycloaddition). This copper-free reaction enables efficient and stable labeling of azide-functionalized biomolecules while preserving native biochemical processes. ADIBO reagents form stable 1,4-disubstituted 1,2,3-triazoles, making them suitable for applications such as cellular imaging and nucleotide functionalization.

By eliminating the need for catalysts, ADIBO reagents are compatible with sensitive biological systems and versatile across various research and diagnostic purposes. This protocol outlines the steps for labeling biomolecules, such as proteins or peptides, using ADIBO dyes through SPAAC.

Product name	λ_{Ex} (nm)	λ_{Em} (nm)	Packing Unit	Catalog number
ICG ADIBO	785	812	1mg / 5mg / 25mg	DOC1061
TAMRA ADIBO	543	575	1mg / 5mg / 25mg	DWR1001
FAM ADIBO	494	522	1mg / 5mg / 25mg	DWF1001
qFlamma® Black01 ADIBO	484	400~800	1mg / 5mg / 25mg	QWD1001
Flamma® 774 ADIBO	774	800	1mg / 5mg / 25mg	DWC1061
Flamma® 749 ADIBO	749	774	1mg / 5mg / 25mg	DWC1031
Flamma® 675 ADIBO	675	694	1mg / 5mg / 25mg	DWC1051
Flamma® 648 ADIBO	649	666	1mg / 5mg / 25mg	DWC1021
Flamma® 581 ADIBO	578	593	1mg / 5mg / 25mg	DWC1415
Flamma® 552 ADIBO	552	567	1mg / 5mg / 25mg	DWC1011
Flamma® 496 ADIBO	496	520	1mg / 5mg / 25mg	DWC1001

Materials and Preparation

- Biomolecule: Azide-functionalized protein or peptide.
- ADIBO Dye
- Buffer: PBS (pH 7.4) or MES (pH 6.0–6.5, optional).
- Solvent: DMSO or DMF.
- Purification Tools: Dialysis membrane, desalting column, or size-exclusion chromatography.

Procedure

1. Prepare Biomolecule:
   - Dissolve azide-functionalized biomolecule in PBS at 1–5 mg/mL.
2. Prepare ADIBO Dye Stock Solution:
   - Dissolve ADIBO dye in DMSO or DMF (10mM stock)
   - Protect from light and use fresh solution.
3. Labelling Reaction

- Add ADIBO dye stock to the biomolecule solution at a molar ratio of 1:5–10 (biomolecule:ADIBO dye).
- Incubate the reaction mixture at 20–25°C for 1–4 hours with gentle agitation.
- For acidic biomolecules, use MES buffer (pH 6.0–6.5) instead of PBS.

Purification

- Remove unreacted dye using one of the following:
  - Dialysis: Recommended for proteins to ensure gentle and thorough purification.
  - Desalting Columns: Suitable for rapid removal of small unreacted molecules, ideal for small biomolecules.
  - Size-Exclusion Chromatography: Provides precise separation for complex samples.
- Verify the purification process using UV-Vis or fluorescence spectroscopy to confirm removal of unbound dye.

Storage

- Store labeled biomolecules at 4°C in PBS, protected from light.
- For long-term storage, lyophilize and store at -20°C.
- Protect all fluorescent dyes from light exposure by using amber vials or wrapping containers in aluminum foil to prevent photodegradation.
- Avoid repeated freeze-thaw cycles to maintain the structural integrity and functionality of labeled biomolecules.

Key Considerations

- Buffer Choice: Avoid using buffers that contain primary amines (e.g., Tris), as they can interfere with the reaction. PBS or MES buffer is typically used.
- Reaction Optimization: The pH and incubation time may need to be optimized depending on the specific biomolecule and labeling efficiency desired.
- Purification: Proper purification is critical to remove unreacted dye and ensure that only the labeled biomolecule is used in subsequent applications.

Note: To maintain structural integrity and achieve efficient labeling, optimize reaction conditions (e.g., pH, time) and, if necessary, perform additional purification to remove unreacted dye or by-products.

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