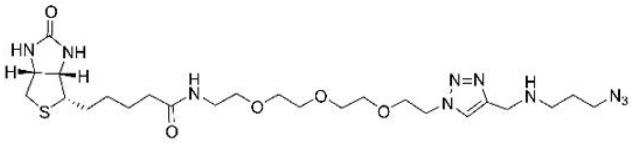
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BIOTIN AZIDE PLUS

SKU: CCT-1488



DESCRIPTION

Azide Plus reagents is the most recent step in improving CuAAC reaction in complex media developed by scientists at Click Chemistry Tools. Azide Plus reagents contain a complete copper-chelating system in their structure, allowing for the formation of strong, active copper complexes that act simultaneously as both reactant and catalyst in the CuAAC reaction. This azide-copper complex reacts almost instantaneously with alkynes under diluted conditions. This unprecedented reactivity in the CuAAC reaction is of special value for the detection of low abundance targets, improving biocompatibility, and is also valuable for any other application where greatly improved S/N ratio is highly desired.

SPECIFICATIONS

CAS Number	N/A
Molecular Weight	582.72
Appearance	Off-white to slightly orange amorphous solid or oil
Chemical Formula	C24H42N10O5S
Purity	>95% (HPLC)
Unit Size	1 mg, 5 mg, 25 mg, 100 mg
Solubility	DMSO, DMF, MeOH
Storage Instructions	-20°C. Desiccate
Shipping Conditions	Ambient temperature
Shipping Instructions	Ambient temperature

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CLICK REACTION PROTOCOL

Click Reaction Protocol for Staining Fixed/Permeabilized Cell

This is a general protocol for fixed/permeabilized cell imaging through a copper-catalyzed click reaction using the fluorescent Azide Plus reagent. We recommend using this protocol as a starting point for optimization of particular click chemistry procedures. We have found that a 1.5-3.0 µM concentration of Azide Plus reagent was optimal for most applications, including imaging of EdU incorporated into newly synthesized DNA and imaging of OPP labeled proteins without causing a high background signal. The optimal final concentration of the Azide Plus reagent is sample dependent and may range from 0.5 µM to 10 µM. Final concentrations below or above this range are also possible, and should be optimized per the specific application.

1. Prepare the following click solutions:
   — 50 mM copper sulfate in water
   — 300 mM sodium ascorbate in water (dissolve 60 mg of sodium ascorbate in 1 mL of water)
   — Subtext
2. 1 mM Azide Plus reagent in DMSO or water

Table 1

Reaction Component	Number of coverslipsor wells of a 96-well plate			
	1 coverslip or 10 wells	**5 coverslips or 50 wells**	**10 coverslips or 100 wells**	**20 coverslips or 200 wells**
Reaction Buffer (Tris)	888 µL	4.4 mL	8.9 mL	17.8 mL
50 mM Copper Sulfate	10 µL	50 µL	100 µL	200 µL
AZDye Azide Plus Solution (2 µM final concentration)	2 µL	10 µL	20 µL	40 µL
Sodium ascorbate	100 µL	500 µL	1 mL	2 mL
Total Volume	1 mL	5 mL	10 mL	20 mL

3. Remove the permeabilization buffer (if used). Wash the cells in each well twice with 1 ml of PBS. Remove PBS.
4. Immediately add 1 mL of the **Reaction Cocktail** to the sample. Evenly distribute the

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reaction cocktail over the sample.

5. **Protect from light**, and incubate the plate for 30 minutes at room temperature.
6. 6Remove the reaction cocktail. Wash each well once with 1 ml of Wash Buffer. Remove the Wash Buffer.
7. Wash each well with 1 mL of PBS. Remove PBS.

Click Reaction Protocol for Cell Lysates Labeling

This is a general protocol for labeling proteins in cell lysate through a copper-catalyzed click reaction using the fluorescent Azide Plus reagent. We recommend using this protocol as a starting point for optimization of particular click chemistry procedures. We have found that a 20 µM concentration of Azide Plus reagent was sufficient to label all alkyne-tagged proteins in the cell lysate without causing a high background signal. The optimal final concentration of the Azide Plus reagent is sample dependent and may range from 5 µM to 50 µM. Final concentrations below or above this range are also possible, and should be optimized per the specific application.

1. Prepare the following click solutions:
   — 100 mM THPTA ligand in water (100 mg of THPTA in 2.3 mL of water)
   — 20 mM copper sulfate in water (dissolve 11.6 mg of copper II sulfate pentahydrate in 2.3 mL of water)
   — 300 mM sodium ascorbate in water (dissolve 60 mg of sodium ascorbate in 1 mL of water)
   — 1 mM Azide Plus reagent in DMSO or water
2. For each protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
   — 50 µL of protein lysate (1-5 mg/mL) in protein extraction buffer
   — 120 µL of Tris buffer
   — 4 µL of Azide Plus reagent stock solution (5 µM final concentration)
3. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.
4. Add 10 µL of 20 mM $CuSO_4$ solution, vortex briefly to mix.
5. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
6. Vortex continuously or rotate end-over-end for 30 minutes at room temperature.
7. Add the labeling reaction to 3 mL of cold (–20°C) methanol, 0.75 ml of Chloroform and 2.1 mL of water. Cool it to –20°C for 1 hour.
8. Centrifuge for 10 minutes at 13,000-20,000 x g, then carefully remove upper aqueous layer without disturbing the interface layer containing proteins.

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9. Add 450 µL of methanol, vortex briefly.
10. Centrifuge for 5 minutes at 13,000-20,000 × g to pellet protein. Carefully remove and discard supernatant.
11. Open the lid to microfuge tube and allow protein pellet to air dry. Do not over dry the pellet!

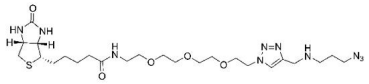
SELECTED REFERENCES

1. Li, B., *et al.* (2020). TMEM132A, a Novel Wnt Signaling Pathway Regulator Through Wntless (WLS) Interaction. *Front Cell Dev Biol.,* **8**, 599890. [PubMed]

DOCUMENTS

- Safety Data Sheet
- Download CoA
- Datasheet

GALLERY IMAGES



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