

Metabolic Labeling of Alkynes using BiotinAz using CuAAC

Prepare stock solutions of the following reagents in PBS.

Reagents	MW (g/mol)	C _{Stock} (in PBS)	
• CuSO ₄	249.70	100mM	(~1 month shelf life)
• Sodium ascorbate	198.11	10mM	(make fresh)
• THPTA	434.50	50mM	(~1 month shelf life)
• BiotinAz	444.55	50mM	(~1 month shelf life)

Prepare stock solutions of the following reagents in PBA.

Streptavidin-PE in PBA 5µg/ml (PBA = 500ml PBS, 5gram BSA, 100mg sodium azide)

Reaction buffer (Prepare freshly and incubate 5min before adding to cells)

Add 10ml PBS

Add 25µl of CuSO₄ stock

Add 20µl THPTA stock

Add 20µl BiotinAz

1. Incubate cells 3d with medium containing sugars
2. Collect cells and wash in 2x PBS
3. Add 95µl reaction buffer (see above)
4. Add 5µl sodium ascorbate (10mM stock in PBS)
5. Incubate 10 minutes at 37°C
6. Wash 3x with PBS + 1% BSA (no sodium azide!!!)
7. Add 0.5µg/ml Streptavidin-PE in PBA (40 µl) for 20min at 4°C
8. Wash 3x with PBS + 0.1% BSA
9. Resuspend in PBS + 0.1% BSA and acquire