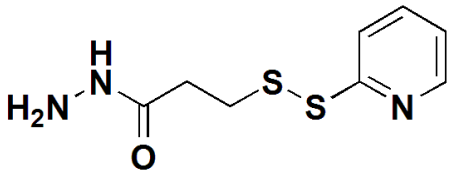
ProteoChem
Crosslinking Protein Biology & Chemistry

PDPH Protocol and Product Information Sheet

Product Category:	Heterobifunctional Crosslinkers
Catalog Number(s):	c1113-100mg, c1113-1g, c1113-custom
Product Name:	PDPH Crosslinker
Alternative Name(s):	3-[2-Pyridyldithio]propionyl hydrazide
CAS Number:	N/A
Chemical Formula:	$C_8H_{11}N_3OS_2$
Molecular Weight:	229.32
Spacer Arm Length:	9.2 Å
Storage:	Upon receipt store at -20°C or lower (shipped at ambient temperature). Protect from moisture (i.e. humidity); blanket under desiccated, inert gas.



General PDPH Crosslinking Protocol

Perform Steps A and B simultaneously

A. Glycoprotein Oxidation

1. Immediately before use, dissolve Sodium *meta*-Periodate (cr8103-5gm) at a concentration of 20mM in 100mM Sodium Acetate, pH 5.5. Make an equal volume of Sodium *meta*-Periodate solution as glycoprotein solution. Store on crushed ice and protected from light until use.
2. Gently mix the glycoprotein solution with the periodate solution (1:1) (0.5mL to 1mL of each is a typical volume).
3. Allow reaction to proceed at 0-4°C for 30 minutes.
4. Dialyze overnight in 100mM Sodium Phosphate 150mM NaCl buffer, pH 7.2 or desalt the oxidized glycoprotein through gel filtration (i.e. spin format) with a resin such as Sephadex® G-25 to remove excess periodate. The desalting column should first be equilibrated with 100mM Sodium Phosphate 150mM NaCl buffer, pH 7.2 to exchange oxidized glycoproteins to the appropriate buffer for conjugation.

B. Sulfhydryl Crosslinking Reaction

1. Prepare previously reduced sulfhydryl-containg protein in 100mM Sodium Phosphate 150mM NaCl buffer, pH 7.2
2. Allow vial of PDPH Crosslinker to fully equilibrate to ambient temperature before opening to prevent condensation inside the vial (PDPH is moisture-sensitive).
3. Immediately before use, dissolve PDPH in DMSO (cr8105-25ml) or DMF (cr8106-25ml) at 25-50mM.
4. Add sufficient PDPH solution to give a molar excess of 5-10 fold molar excess of PDPH to protein. Note: Do not exceed 10% DMSO or DMF in final reaction mixture.
5. Allow crosslinking reaction to proceed for 2 hours at room temperature (≥ 4 hours at 0-4°C).
6. Remove excess reagent by dialyzing overnight in 100mM Sodium Phosphate 150mM NaCl buffer, pH 7.2 or desalt the crosslinked protein through gel filtration (i.e. spin format) with a resin such as Sephadex® G-25 to remove excess PDPH. The desalting column should first be equilibrated with 100mM Sodium Phosphate 150mM NaCl buffer, pH 7.2 to exchange PDPH-activated protein to the appropriate buffer for conjugation.

C. Protein Conjugation

1. Combine PDPH-activated protein and oxidized glycoprotein from steps A and B in appropriate ratios for the desired conjugation and number of functional groups available.
2. Allow the samples to react at room temperature 2-3 hours. React slightly longer if reaction must be done on ice.
3. Optional: Conjugate can be isolated from unconjugated proteins by size exclusion or ion exchange chromotography and can also be analyzed by SDS-PAGE.

References:

Hermanson, G.T. 1996. Bioconjugates Techniques. Academic Press, San Diego, CA USA.

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