

# DPDPB

## 1,4-di-(3'-[2'-pyridyldithio]propionamido)butane

21702

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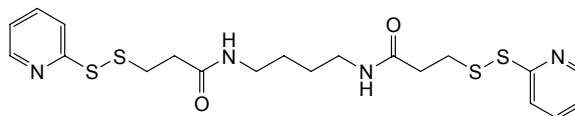
**Number**

21702

**Description**
**DPDPB** (1,4-di-[3'-(2'-pyridyldithio)propionamido]butane), 50 mg

Molecular Weight: 482.71

Spacer Arm: 19.9 Å


**Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature.

**Introduction**

DPDPB (1,4-di-[3'-(2'-pyridyldithio)propionamido]-butane) is a homobifunctional sulfhydryl-reactive crosslinker. The dithiopyridyl groups react with sulfhydryl groups via a disulfide interchange liberating two molecules of pyridine-2-thione and producing a crosslink containing two cleavable disulfide bonds. DPDPB is insoluble in aqueous buffers and is first dissolved in organic solvents, such as DMSO (Table 1). DPDPB has two absorbance maxima, 237 nm and 287 nm at wavelengths from 350 to 200 nm with extinction coefficients of  $1.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  and  $8.8 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ , respectively.<sup>1,2</sup> Pyridine-2-thione has absorbance maxima at 272 nm and 344 nm and the extinction coefficient at 343 nm is  $8.08 \pm 0.3 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>3</sup> This demonstrates that pyridine-2-thione is released upon reduction of DPDPB and indicates that the reaction can be monitored by measuring its absorbance at 344 nm.<sup>1,2</sup>

**Table 1.** DPDPB solubility in various solvents.

Solvent	Concentration	
	(mg/ml)	(mM)
Water	-	-
Acetonitrile	~4	8
Methanol	> 42	> 87
DMSO	> 44	> 91
DMF	> 38	> 79
Dioxane	~ 9	20
Ethanol (HPLC grade)	~14	28

**Example Procedure for Crosslinking Reduced IgG and  $\beta$ -Galactosidase<sup>4</sup>**

In this method, whole IgG is reduced with 2-MEA, which cleaves disulfide bonds between the antibody heavy chains while preserving the disulfide bonds between the heavy and light chains. During reduction, the absolute concentration of 2-MEA is more critical than antibody concentration as 1-10 mg IgG can be effectively reduced with 50 mM 2-MEA. To prevent metal-catalyzed oxidation of sulfhydryls, EDTA is included in buffers. The protocol can be modified for other proteins, peptides and molecules.

**A. Additional Materials Required**

- DPDPB stock solution: 10 mg/ml DPDPB in DMSO
- 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408)
- 0.5 M EDTA
- 1 M Sodium phosphate, pH 6.0
- Phosphate-buffered saline (e.g., 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, Product No. 28372) or other buffer at pH 6.5-7.5
- 37°C incubator or water bath
- Desalting column, such as Dextran Desalting Columns (Product No. 43230)

## B. Material Preparation

Reducing Buffer	Prepare 1 ml of buffer by combining 100 µl of 1 M sodium phosphate pH 6.0, 5 µl of 0.5 M EDTA and 900 µl ultrapure water.
Conjugation Buffer	Add 20 µl of 0.5 M EDTA to 10 ml of PBS for each 10 ml of Conjugation Buffer required.
IgG Solution	Dissolve 2.5-5.0 mg IgG in 1 ml of Reducing Buffer.

## C. Reduction of IgG Disulfide Bonds

1. Add the 1 ml IgG Solution to a 6 mg vial of 2-MEA and gently shake vial to dissolve.
2. Incubate reaction for 90 minutes at 37°C.
3. Cool the solution to room temperature.
4. Remove 2-MEA from the reduced antibody using a desalting column equilibrated with Conjugation Buffer. After the antibody solution has entered the resin bed, add additional Conjugation Buffer and collect 500 µl fractions.

**Note:** The antibody generally emerges when one void volume of buffer has been added to the column after adding the antibody. Molecules smaller than the column's exclusion limit, such as 2-MEA, emerge from the column in subsequent fractions, which can be discarded after confirming that all fractions containing protein have been collected.

5. Determine antibody location by measuring for fractions having peak absorbance at 280 nm. Pool fractions containing reduced antibody and adjust volume to 2.5 ml with Conjugation Buffer. Proceed immediately to Section D to minimize sulfhydryl oxidation.

## D. Reaction of DPDPB with Reduced IgG and β-Galactosidase

1. Add 100 µl of DPDPB stock solution to the peak fraction of reduced IgG (~ 5 mg). Allow the DPDPB and the reduced IgG to react for 30 minutes at 30°C.
2. Apply the mixture to a 5 ml desalting column equilibrated with PBS. Collect 1 ml fractions. Monitor the absorbance of the fractions at 280 nm and pool the protein-containing fractions.
3. Add the pooled fractions to 2 mg of β-D-galactosidase. Incubate the reaction for 1 hour at 30°C.
4. Apply the mixture to a desalting column equilibrated with PBS. Collect 1 ml fractions. Monitor the absorbance at 280 nm. Pool the fractions containing the conjugated protein.

## References

1. Zecherle, G.N. (1990). Doctoral dissertation, University of California – Davis.
2. Traut, R.R., *et al.* (1989). Crosslinking of protein subunits and ligands by the introduction of disulfide bonds in Protein Function-A Practical Approach, Creighton, T.E. ed., IRL Press at Oxford University, Oxford, pp. 101-133.
3. Stuchbury, T., *et al.* (1975). Reporter groups delivery system with both absolute and selective specificity for thiol groups and an improved fluorescent probe containing the 7-nitrobenzo-2-oxa-1,3-diazole moiety. *Biochem J* **151**:417-32.
4. O'Sullivan, M., *et al.* (1979). Comparison of two methods of preparing enzyme-antibody conjugates: application of these conjugates for enzyme immunoassay. *Anal Biochem* **100**:100-8.

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