DNP-NHS labeling reagent

2,4-dinitrophenyl-N-Hydroxysuccinimide

For research use only.

Not for use in therapeutic or diagnostic procedures.

Storage temperature : 2-8°C

Packaging details :

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>65DNSABD</td>
<td>0.2mg</td>
</tr>
<tr>
<td>65DNSABB</td>
<td>1mg</td>
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</tbody>
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1. Background

DNP (2,4-Dinitrophenyl) is a small organic motif which has been widely used for the labeling of oligonucleotides or peptides during synthesis. It has been shown to be an excellent complementary tag in molecular biology for applications such as DNA hybridization or the detection of PCR amplified products. Moreover, the reduced size of the motif enables the detection of very small entities without steric hindrance drawbacks often encountered with larger tags. For instance, double labeling of peptide substrate with DNP and biotin was successfully carried out for the study of proteolytic cleavage with caspase 3.

Several reagents have been developed for the labeling of biomolecules and their subsequent detection. DNP-NHS (DNP-N-hydroxysuccinimide ester) has been synthesized to enable the post-synthesis labeling of peptides, oligonucleotides or proteins. The dinitrophenylation procedure is extremely straightforward and target primary amines, similarly to biotinylation with corresponding NHS derivatives.

DNP can be detected using mouse monoclonal antibody 265.5. This mouse IgG1 was raised against DNP-BSA and exhibited high affinity for DNP-aminocaproate as well as for other DNP derivatives. Anti-DNP conjugates were also shown to bind to a wide variety of DNP-labeled compounds: proteins such as DNP-thyroglobulin, peptides like DNP-substrate for CPP32 and DNP labeled oligonucleotides.

References


2. Reagent description

- **Formula :** C₁₆H₁₈N₄O₈
- **Molecular weight :** 394
- **Absorbance peaks :** 205 – 280 – 360 nm
- **Molar absorption coefficient at 360 nm** (ε₃₆₀ₙ₉) : 17,500 cm⁻¹M⁻¹
- **Rz ratio = OD₃₆₀nm/OD₂₈₀nm** in aqueous conditions = 3.43
- **Properties :** yellow crystals
- **Solubilization :** clear yellow solution in acetonitrile or DMSO after thorough vortexing ([C] = 5 mg/mL).

Based on an average initial labeling molar ratio (DNP:compound) of 10:1, and on a compound molecular weight of 40,000 Da, each vial from the two available sizes enables the labeling of 2 and 10 mg of compound, respectively.

3. Dinitrophenylation principle
4. Labeling procedure

4.1. Guidelines and reagent preparation

Reconstitute desiccated DNP-NHS with acetonitrile or DMSO in order to obtain a 2.5 mg/mL solution. Vortex thoroughly until the complete dissolution of crystals.

Compounds to be labeled (proteins, peptides, oligonucleotides) should be dialyzed against or diluted with a freshly prepared labeling buffer: 100 mM carbonate buffer pH 9.0. Should the target molecule be underlabeled (too few DNP per target), the use of a less basic buffer (e.g. 100 mM PO₄ pH 8.0) may improve the labeling efficiency. Ideal compound concentration for labeling ranges from 1 to 5 mg/mL.

Depending on the nature of the compound to be labeled, DNP-NHS should be added in a 5 to 15 fold excess.

4.2. Experimental procedure (example)

- Dialyze 1 mg of protein, MW 40,000 Da (eq. 25 nmoles), against the labeling buffer. Check protein concentration and adjust it to 1-5 mg/mL if necessary.
- Proceed to 4 successive 10 µL additions of DNP-NHS at 1mg/mL (eq. 250 nmoles). Let the solution stand for about 2 minutes in between steps.
- Leave the solution under gentle stirring for 1 hour at room temperature.
- Purify the mixture on G25 SF column to remove unreacted DNP-NHS.
- Final molar ratio (number of DNP per protein) can be approximated by making an absorbance spectrum of the recovered fraction and by the formula given beside.
- Using the characteristic Rz value specified in the product’s datasheet, this calculation takes into account the absorption of DNP at 280 nm.

4.3. MRf calculation

Periodic absorbance measurement in the 350-400 nm range gives the Rz value, which is indicative of the number of DNP per protein.

\[ MRf = \frac{OD_{360nm}}{\varepsilon_{DNP}} \frac{\varepsilon_{compound}}{Rz} (OD_{360nm} - \frac{OD_{360nm}}{Rz}) \]

5. Storage conditions and stability

- The desiccated product can be kept at 2-8°C for at least 2 years.
- Once solubilized in acetonitrile or DMSO, DNP-NHS can be stored for at least two days at 2-8°C without any alteration of its reactivity.
- The solution can also be frozen at –80°C in a sealed tube for longer term storage and can be submitted at least to two freeze/thaw cycles.