

## Amino - NHS ester labeling Protocol

This protocol is for labeling of oligonucleotides carrying an amine group with mono-NHS ester dyes.

This protocol is based on Rahul's and Jeehae's and modified by SH. Thanks.

The key concepts are

- 1) The optimal **amine:dye ratio is 1:5** (I prefer to use **1:20**)
- 2) Set **pH of reaction buffer to 8.5** (High pH enhances the acylation rate and hydrolysis of the esters)
- 3) Typical amine concentration in reaction buffer is about 150uM. (but not critical)

### 1. Preparation

\* **Reaction buffer** (100mM Sodium tetraborate)

Dissolve 201 mg of sodium tetraborate anhydrous (or 380 mg for decahydrate) in 10ml dewater. (use fresh bufer) Adjust the pH to 8.5 with HCl. (usually need ~70ul of 12M HCl. Initially is at pH9.2)

\* **Dye** (final: 23mM in DMSO)

Dissolve 1mg of mono-NHS ester dye in 56 ul of DMSO. Dye in DMSO can be stored in -20 for 2 weeks.

\* **Oligo** (final concentration: 200uM in reaction buffer, 100mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> pH8.5)

ex. If you have 10 nMole of oligos, add 50ul of labeling buffer.

### 2. Reaction

Mix followings. The total amount can be varied but keep the ratio of contents. (NH<sub>3</sub>:dye=1:20)

- | 14ul of 22 mM dye in DMSO => 1.5 mM
- | 75 ul of 200 uM oligo carrying on amine group => 150 uM
- | 11ul of diWater

### 3. Incubation

Incubate at RT for 6 hours on a gently shaking mixer in dark.

### 4. EtOH precipitation

- a) Add 1/10 of reaction solution volume of 3M NaCl to reaction solution. Add 2.5 volume of reaction solution of cold (-20 chilled) absolute ethanol. Mix well and incubate in -20 for 30mins.
- b) DNA precipitation results in truning the solution turbid. Centrifuge the solution at --12,000g for 30mins to recover the DNA as a pellet.
- c) Remove the supernatant and wash the pellet 3-4 times with 70% etOH.

### 5. Redissolve the DNA according to your aplication.

### 6. Labeling efficiency

Cy3 and Cy5 molar extinction coefficients are 150,000 and 250,000 M<sup>-1</sup>cm<sup>-1</sup>. Dye absorptions at 260nm are less than 10%.