GLEN RESEARCH

part of Maravai LifeSciences

Pre-Synthesis Labeling of Amino-Modifier CPG

(See Also: Technical Bulletin - Methods to Avoid Inactivation of Primary Amines)

In many instances it is more efficient to label amino-modifier CPG with a chromophore before or immediately after oligonucleotide synthesis. Incorporation of an amine reactive label using solid phase chemistry in a non-aqueous solvent is more efficient and requires a lower excess of label.

Materials:

- Amino-Modifier C7 CPG, Glen Research 20-2958 or 3'-Amino-Modifier Serinol CPG, Glen Research 20-2997
- Diisopropylethylamine (DIEA), Aldrich 38,764-9.
- Dimethylformamide (DMF), Aldrich 22,705-6 or equivalent.
- Piperidine, Aldrich 41,102-7 or equivalent.
- Amine reactive label (TAMRA-NHS, Dabsyl-Cl, etc.)

Procedure:

1. Removal of Fmoc amino protection with 20% piperidine/DMF. Prepare a 20% v/v solution of piperidine in DMF.

    - Flush a synthesis column of amino-modifier CPG (1µmole) with 2.5 ml of 20% piperidine/DMF solution and leave for 2-5 minutes. This can be done with two disposable syringes.
    - Repeat above procedure 2-3 times with fresh piperidine solution. (Note if you want to quantify Fmoc removal collect piperidine solution washes, dilute to 10 ml with 20% piperidine/DMF and read absorbance at 301 nm vs. 20% piperidine/DMF. E-301= 7.8 ml/µmole-cm.)

- Rinse CPG column X 3 with 3 ml DMF. This can be done using syringes.
- Repeat using acetonitrile. This can be done on the synthesizer or using syringes.
- Dry column using Argon gas.

2. Labeling amine with amine reactive label. (Labeling can also be done in column) Transfer support to 1.5 ml polypropylene tube.

    - Add 140 µl DMF.
    - Add 10 µl DIEA (56 µmole) and gently mix.
    - Dissolve 10 µmole label in 100 µl DMF. Lower molar ratio can be used.
    - Add to support and incubate for 1-2 hour at RT with occasional mixing.
    - Spin down CPG in microcentrifuge and carefully remove solution from CPG with Pasteur pipette.
    - Wash CPG with 1 ml DMF. You can use a microfuge to pellet CPG, remove supernate with pipette. Repeat 3 times.
    - Wash CPG with ACN, 3 times as above.
    - Wash CPG with DCM, 3 times as above.
    - Air dry CPG and transfer to synthesis column for synthesis. (Note some free label may wash off the column during the first synthesis cycle but this will not affect the synthesis)

3. Determination of loading efficiency. Transfer 5 mg of support to a cleavage vial (polypropylene tube or glass vial).

    - Add 1 ml concentrated NH4OH (or 1 ml 50 mM K_2CO_3 in anhydrous MeOH for base labile labels) and incubate for 2-4 hour at RT.
    - Transfer supernate to a 10 ml volumetric flask.
    - Rinse CPG with H_2O and combine in 10 ml volumetric flask. If label is very hydrophobic it may be necessary to rinse CPG with MeOH to extract labeled linker.
    - Adjust volume to 10 ml with H_2O (or MeOH) and read absorbance at the labels λmax vs. H_2O (or MeOH).
    - Calculate label concentration using extinction coefficient of label.
    - Compare value with reported CPG loading.
    - Note: The value determined for label conjugation is typically 80-90% that based on DMT assay. The DMT cation assay is quantitative. However, the label assay is dependent on the efficiency of hydrolysis from the support typically 80-90%.