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Solid Phase Oligo COUPLE-IT™ Kit
Instruction Manual

Product:	Solid Phase Oligo COUPLE-IT™ Kit (2 Reactions)
Product No:	CPL0002
Procedure:	Coupling of primary amino modified nucleic acids to magnetic solid supports (16.7)
Kit Storage:	4°C
Stability:	Performance of this kit is guaranteed for 6 months from date of purchase

The Solid Phase Oligo COUPLE-IT™ Kit enables convenient covalent coupling of primary amino-modified oligonucleotides and other amino-modified nucleic acids to Magnetic Porous Glass (MPG®) supports. Oligo-coupled-MPG® can be used for solid phase nucleic acid-based applications including: *in vitro* translation; subtractive hybridization; immobilized cDNA libraries; hybridization-based assays; and affinity purification of nucleic acid binding proteins.

Amino-modified substrates used for coupling can be generated by either synthesizing the oligonucleotide with an amino modifier phosphoramidite which puts an amine group on the 5' terminus of the molecule, or incorporation of aminoallyl-dUTP or aminoallyl-UTP using DNA or RNA polymerase respectively.

The kit contains all the reagents and MPG® solid support needed to carry out two efficient coupling reactions (10 mg MPG® per reaction). The resulting product is an oligo-coupled magnetic solid support which can be stored for future use and is reusable.

Kit Contains:

1 ml	MPG® LCA (20 mg/ml)
3.0 ml	2X Coupling Buffer
150 µl	Activation Solution
4 x 5 mg	Reducing Agent
1 ml	Blocking Buffer
4.0 ml	Wash Buffer
2.0 ml	Storage Buffer

Materials: (not included with the kit):

Nucleic Acid to be Coupled
Magnetic Particle Separator
Ethanol
Ammonium Acetate or Sodium Acetate
Molecular Biology Grade Glycogen (optional)
Nuclease-free Water
Low Speed Rotator or other End-Over-End Mixer

AMINO-MODIFIED NUCLEIC ACID

The nucleic acid must contain a primary amino group somewhere in the sequence. For many downstream applications it is preferable to have the primary amino group at the 5' terminus. This can be accomplished by synthesizing the oligonucleotide with a 5'-amino modifier. We have found that best coupling yields are obtained using the 5'-amino-modifier C6-TFA from Glen Research (Cat. No.: 10-1916-xx).

If the nucleic acid has not been purified by either gel or HPLC, it is recommended to either gel purify, or perform a phenol extraction.

PREPARATION OF AMINO-MODIFIED NUCLEIC ACID FOR COUPLING

If the amino-modified nucleic acid is in solution, carry out the following steps to ethanol precipitate the nucleic acid. If it is in lyophilized form, first resuspend in nuclease-free water.

1. Determine the concentration in pmol of the nucleic acid solution. Concentration of nucleic acids can be determined by measuring the absorbance at 260 nm (A_{260}) using a UV spectrophotometer.

   Conversion of A_{260} to µg

For oligonucleotide	1.0 OD A_{260} = 30 µg
For RNA or ssDNA	1.0 OD A_{260} = 40 µg
For dsDNA	1.0 OD A_{260} = 50 µg

   Conversion of µg to pmol

   $$\text{pmol of nucleic acid} = \frac{(\mu g \times 10^{-6})\,(1 \times 10^{12}\ \text{pmol/mol})}{(330\ \text{g/mol})\,(\#\ \text{bases})}$$

2. Pipette approximately 25-30 nmol of the nucleic acid solution into a microcentrifuge tube.
3. Add to the nucleic acid solution either 5 M ammonium acetate to a final concentration of 2.5 M; or, 3 M sodium acetate to a final concentration of 0.3 M; either salt works equally well. Add 3 volumes ice cold ethanol and Incubate on dry ice or in a -70°C freezer for 15-30 minutes
4. Centrifuge at 12,000 g for 10-15 minutes at 0-4°C. Carefully remove the supernatant.
5. Wash the pelleted nucleic acid with 0.5 ml ice cold 70% ethanol and centrifuge at 12,000g for 30 seconds to 1 minute. Remove supernatant and air dry or speed vac.
6. Prepare 1X Coupling Buffer by pipetting 1.5 ml of the 2X Coupling Buffer provided into a fresh nuclease-free tube. Add 1.5 ml of nuclease-free water. Vortex to thoroughly mix solution. (NOTE: This solution is stable at room temperature and is used for both the activation and coupling steps where indicated below.)
7. Resuspend pelleted amino-modified nucleic acid in 100 µl 1X Coupling Buffer

ACTIVATION

1. Gather the following items for use at room temperature:

   MPG® LCA,
   1X Coupling Buffer (prepared in *step 6 PREPARATION OF AMINO-MODIFIED NUCLEIC ACID FOR COUPLING*)
   Activation Solution
   1 Tube - Reducing Agent (to be used in the COUPLING STEPS)

2. Gently vortex the tube of MPG® LCA to suspend the particles. Remove 10 mg (500 µl) of MPG® LCA and place in a nuclease-free microcentrifuge tube. Magnetically separate the particles from the buffer by placing the tube in a magnetic separator. Carefully remove the supernatant with a micropipettor.

3. Add 190 µl of 1X Coupling Buffer and 64 µl of Activation Solution to the particles. Remove the tube from the magnetic separator, gently vortex to mix the components and place the tube on a low speed rotator or other end-over-end mixer. Incubate at room temperature for 1.5 hours with gentle rotation, keeping the solution mixed during the incubation.
4. Remove the tube from the rotator or other end-over-end mixer and place in magnetic separator to magnetically separate the particles. Carefully remove the supernatant.
5. Wash the particles by adding 250 µl of 1X Coupling Buffer and mix by gently vortexing. Magnetically separate and carefully remove the supernatant. Repeat this washing step 4 more times. The activated particles are now ready for nucleic acid coupling.

COUPLING

1. Prepare Reducing Agent solution by adding 500 µl of 1X Coupling Buffer to the tube containing the Reducing Agent. Vortex to mix well.
2. Add the 100 µl of the Coupling Buffer containing the ~ 25 nmol of the 5'-amino modified nucleic acid (prepared in step 7 of *PREPARATION OF AMINO-MODIFIED NUCLEIC ACID FOR COUPLING)* to the activated particles. Add 10 µl of Reducing Agent solution and gently vortex to mix contents of tube.
3. Incubate overnight at room temperature on a low speed rotator or other end-over-end mixer. The particles must be mixed constantly during incubation to ensure efficient coupling.
4. Remove the tube from the rotator or other end-over-end mixer and magnetically separate the nucleic acid-coupled particles. Carefully remove the supernatant.

BLOCKING

1. Gather the following items for use at room temperature:

   Blocking Buffer
   1 tube of Reducing Agent
   Wash Buffer
2. Prepare fresh Reducing Agent solution by adding 500 µl of 1X Coupling Buffer to the tube containing the Reducing Agent. Vortex to mix well.
3. Add 450 µl of Blocking Buffer and 50 µl of the freshly made Reducing Agent solution to the oligo-coupled particles. Vortex gently to mix and incubate at room temperature on a low speed rotator or other end-over-end mixer for 2 hours.
4. Remove the tube from the rotator or other end-over-end mixer and magnetically separate the particles. Carefully remove the supernatant.
5. Wash the particles by adding 250 µl of Wash Buffer and mix by gently vortexing. Magnetically separate and carefully remove the supernatant. Repeat this washing step 4 more times. The nucleic acid-coupled MPG® is now ready for downstream applications.
6. The nucleic acid-coupled magnetic particles can be stored at 4°C in 100 µl Storage Buffer.

ASSESSMENT OF COUPLING EFFICIENCY

The efficiency of the coupling is dependent on the size of the nucleic acid being coupled and the availability of primary amino groups. Nucleic acids with a terminal amino group will couple more efficiently than nucleic acids with internally incorporated primary amines. Increasing the length of the nucleic acid will decrease the efficiency of coupling. Typically, for 20-25 nmol of a 30'mer containing a 5' amino linker, the coupling efficiency is between 12-20%. Coupling efficiency can be determined by measuring the A_{260} of the coupling reaction mix before and after the coupling reaction.

TROUBLE SHOOTING

1. The nucleic acid solution must be free of amines since these compounds will compete with the 5'-amino-modified nucleic acid for reactive sites on the particle. Therefore, the amine-containing protecting group salts present in crude oligo preps must be completely removed for efficient coupling (see recommendations on page 2 for proper oligo preparation). Also, buffers containing Tris (i.e. TE Buffer) will inhibit the coupling reaction. The nucleic acid solutions can be desalted either by ethanol precipitation or by using a gel-filtration spin column. If using a spin column do not trade purity for yield, (i.e. use minimal loading volumes).
2. The length of the nucleic acid will affect the efficiency of coupling.
3. For downstream applications such as PCR or hybridization based assays, the coupled particles must be equilibrated with the appropriate reaction buffer. This is accomplished by washing the particles 3 times with 1 ml reaction buffer per 10 mg particles.

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