SPHERO™ Technical Note **STN-19 Rev A 021220**

COVALENT COUPLING OF T25 DNA TO CARBOXYL MAGNETIC PARTICLES

T25 DNA Coated NanoParticles and Microspheres

- Used for the detection and identification of oligonuceotides
- Allow for simple, rapid and reliable binding of Biotinylated PCR hybridized with a poly(dA)-tailed oligo

MATERIALS:

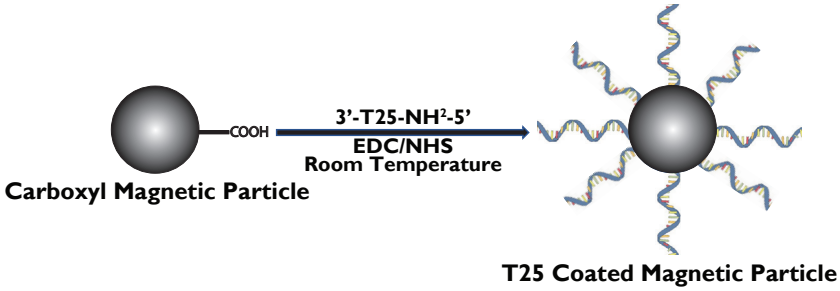
1. SPHERO™ Carboxyl Magnetic Particles (Cat. No.: CM-05-10H, High Iron, 1.0%w/v, 0.4-0.69µm, 10mL)
2. 0.05M MES buffer, pH 5.5
3. N-(3-Diethlaminoropyl)-N'-ethylcarbodiimide (EDAC)
4. N-hydroxysuccinimide (NHS)
5. Carbonate Buffer, 0.1M, pH 8.0
6. NH^2-T25 DNA
7. Phosphate Buffered Saline, 0.1M, pH 7.4 (PBS)

EQUIPMENT:

1. SPHERO™ FlexiMag Separator Jr. (Cat. No.: FMJ-1000)

COVALENT COUPLING PROCEDURE:

1. Magnetically separate 10mL of Carboxyl Magnetic Particles and wash 2x with MES buffer
2. Resuspend the particles in 10mL of MES buffer
3. Dissolve 100mg of N-(3-Diethlaminoropyl)-N'-ethylcarbodiimide (EDAC) in 500µL of MES buffer
4. Dissolve 100mg of N-hydroxysuccinimide (NHS) in 500uL of MES buffer
5. Add 500µL of EDAC first to the 10mL of magnetic particles followed by 500µL of NHS solution
6. Rotate the particles mixture at room temperature for 30 minutes
7. Magnetically separate the particles and wash 2x with carbonate buffer
8. Resuspend the particles in 10mL of carbonate buffer
9. Add 167µL of 600µM NH^2-T25 DNA to the particles and rotate the particles mixture at room temperature overnight; the final concentration of the NH^2-T25 DNA in the solution is 10µM
10. Separate the particles magnetically and remove the supernatant
11. Resuspend the particles in 10mL of PBS buffer
12. Magnetically separate and remove the supernatant
13. Resuspend in 5mL of PBS buffer
14. T25 coupled magnetic particles are ready for analysis.



A schematic representation of the covalent coupling method for 3'-T25-NH2-5' DNA to Carboxyl Magnetic Particles using EDC/NHS

DETERMINATION OF THE BINDING CAPACITY FOR T25 COATED MAGNETIC BEADS

MATERIALS:
1. 300uM A25-FAM DNA
2. 2.5M NaCl
3. 1x PBS, pH 7.4 buffer

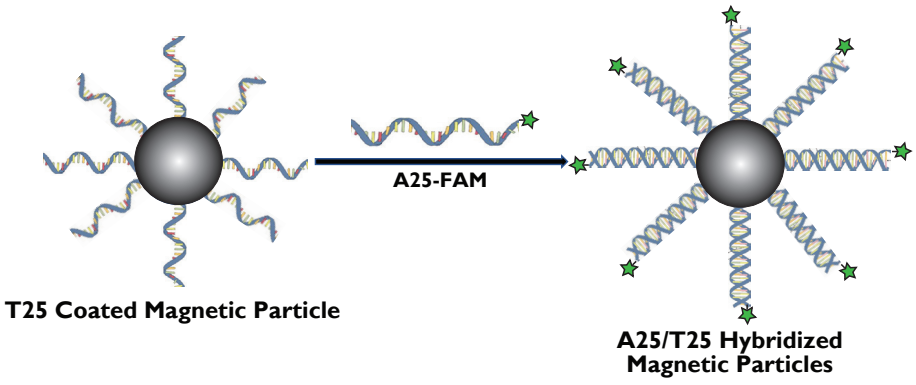
EQUIPMENT:
1. SPHERO™ FlexiMag Separator Jr. (Cat. No.: FMJ-1000)
2. Thermo Scientific™ NanoDrop 2000
3. Eppendorf® Thermomixer®

HYBRIDIZATION PROCEDURE:
1. Preparing 2mL of 10μM A25-FAM DNA in 0.3M NaCl by mixing 67μL of 300μM A25-FAM DNA, 240μL of 2.5M NaCL and 1693μL of 1x PBS
2. Add100μL of T25 Coated Magnetic Particles to a microcentrifuge tube
3. Magnetically separator the T25 Coated Magnetic Particles for 10 minutes. Remove the PBS buffer supernatant
4. Wash the T25 Coated Magnetic Particles 3x with 100μL of PBS and vortex
5. After the third wash, add 100μL of 10μM A25-FAM DNA to the T25 Coated Magnetic Particles
6. Vortex the particles to mix thoroughly
7. Place mixture on the Thermomixer® for three hours at 20°C protected from light.
8. After two hours, separate the particles using the SPHERO™ FlexiMag Separator Jr.

MEASURING THE CONCENTRATION OF HYBRIDIZED OLIGO:
1. Set the wavelength reading to 260nm on the Thermo Scientific™ NanoDrop
2. Collect the Blank Absorbance Value with PBS
3. Measure 2μL of the 10μM A25-FAM DNA solution and record the reading as the Control Absorbance Value
4. Clean probe with 2μL of Milli-Q water and wipe off moisture
5. Measure 2μL of supernatant solution from T25 beads after the hybridization procedure and record the reading as the Experimental Value
6. Calculate the Absorbance Value of the oligo hybridized on the T25 loaded surface by subtracting the Experimental Absorbance Value from the Control Absorbance Value:

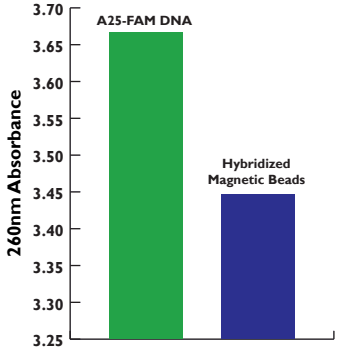
Absorbance Value of Oligo Hybridized = Control Absorbance Value - Experimental Absorbance Value

7. Calculate the molar concentration of A25 DNA hybridized to the T25 coupled magnetic particles by the formula: **(Absorbance of the Oligo Hybridized x 10μM) / (Control Absorbance Value)**



A schematic representation of the hybridization of A25-FAM DNA to 3'-T25-NH2-5' DNA Magnetic Particles

Reduction of A25-FAM DNA using T25 Magnetic Beads and Hybridization Procedure



Sample	260nm Absorbance	Molar Concentration
A25-FAM DNA	3.70	10.0μM
Hybridized Magnetic Beads	3.50	9.29μM

* Binding capacity of the T25 Magnetic Bead was deteremined to be 710nM of A25-FAM DNA/mg of beads

IMPORTANT NOTE: This protocol is for the coupling of T25 oligos and quantitation with A25-FAM oligo. The coupling effiency of other oligos will vary depending on the length of the oligo, coupling conditions, and pH of coupling buffers. Since the quality of the coated particles depends on the quality of reagents and on the coating procedures, high quality reagents should be used while optimizing the coating conditions. As a result of Spherotech's lack of control over the reagents and coating condition, Spherotech can not guarantee the quality or performance of the coated particles even if the provided procedures are followed.