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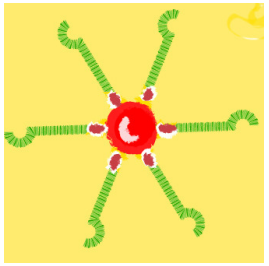
Aptamer 2-step conjugation protocol (EMD Adaptation)



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Protocol status: Working
We use this protocol and it's working

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Abstract

The following protocol consist on an aptamer adaptation from Merck for Antibody conjugation protocol to carboxyl modified microparticles.

Materials

MATERIALS

500mg Sulfo NHS (N-Hydroxysulfosuccinimide) **G-Biosciences Catalog #**BC97

EDC N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**39391-10ML

- PBS Buffer pH =7.4
- PBS-T Buffer (Tween-20 0.01 %) pH=7.4
- MES Buffer 50 mM pH= 6
- Glycine 50 mM aqueous solution
- Storage Buffer : Tris-HCl 50 mM pH = 8 + 0.5 % BSA
- 5' Amino-terminal modified capture aptamer, resuspended in distilled water at 100 µM concentration.

-100-1000 µL Micropipette
- 2-20 Micropipette
- 1000 µL Micropipette tips
- 200 µL Micropipette tips
- Eppendorf Tubes (1.5 mL)
- Thermoblock
- Microfugue
- Ice

Reactives Preparation

1 Prepare a 200 mM aqueous solution of sulfo-NHS. Weight 22.1 mg of sulfo-NHS and dissolve it in 500 µL of distilled water. Keep stored at 4 ºC. Sulfo-NHS can be stored under refrigerator for a couple of months.

2 Prepare 200mM EDC solution. Pipette 22 µL of 97% EDC in 484 µL of distilled water. EDC solutions must be stored at -20 ºC.

3 Prepare a 8 µ dilution of the 5' Amino terminal modified aptamer. Mix 8 µL of 100 µM DNA stock with 92 µL of distilled water.

4 Reconstitute the aptamers by pipetting 100 µL of 8µM dolution on an empty eppendorf tube. Place in a thermoblock at 95 ºC for 10 minutes. And move the tube quickly to an ice bath, letting them to cool down for 10 minutes more. The reconstituted aptamer can be kept at 4ºC for several weeks, or store freezed at -20 ºC for long periods.

Latex Beads Preparation

5 Dilute 200 µL of 2.5 % wt beads stock with 300 µL of additional MES buffer. Reaching a final volume of 500 µL.

6 12000 rpm , 5 minutes the tube at 15.000 rpm for 4minutes. Discard the supernatant and resuspend them in 500 µL of MES buffer.

For beads resuspension, repeated pipetting it's highly recommended, aspiring and blowing out in the eppendorf tube. It's crucial assuring perfect beads disperssion, if little agreggates are appreciated, try reducing centrifugation times or sonicating the beads for resuspension (5 minutes sonication at moderate power).

7 Repeat step 6 twice, resuspending the last time in 1000 µL of MES buffer.

tivaLatex Beads ActivationLatex Beads A

8 Pipette 120 µL of freshly prepared sulfo-NHS 200 mM aqueous solution and add to the latex beads tube.

9 Pipette 12.5 µL of freshly prepared EDC 200 mM aqueous solution and add to the latex beads tube.

10 Incubate at room temperature the tube for 30 minutes under mild agitation conditions. Rotatory wheel agitator is recommended, however, intermitent vortexing, sonication, orbital agitator or balance shaker can be also used.

11 After the incubation time, 12000 rpm , 5 minutes and discard the supernatant. Resuspend the beads in 1000 µL of MES buffer.

Repeat twice more that washing step, but in the final resuspension, add just 300 µL of MES buffer instead of 1000 µL.

Latex Beads Conjugation

12 Add 200 µL of the reconstituted 8 µM aptamer solution, prepared previously in step 4.

13 Incubate the eppendorf tube at room temperature for 2 hours an 30 minutes, under mild agitation.

14 After the incubation time, pipette 100 µL of Glycine 50 mM aqueous solution, for crosslinking activated groups deactivation. Wait for 5 minutes.

15 12000 rpm , 5 minutes and discard the supernatant. Resuspend the beads in 1000 µL of blocking buffer.

Repeat twice more that washing step, but in the final resuspension, add just 500 µL of MES buffer instead of 1000 µL.

16 Conjugated latex beads are at 1% wt concentration related to latex beads in the suspension. Beads can be stored in blocking buffer at 4ºC for some weeks.