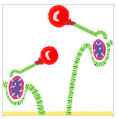

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Aptamer 1-step conjugation protocol

Jorge Fernández[1]

[1]Universidad Complutense de Madrid

1 Works for me dx.doi.org/10.17504/protocols.io.8h4ht8w

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Jorge Fernandez Mendez
Universidad Complutense de Madrid

ABSTRACT

Adaptated version of the crosslinking bioconjugation protocol depicted in https://doi.org/10.1177/1087057106292138 publication.

The following protocol depicts the steps to follow for proper crosslinking of amino modified DNA aptamers with carboxyl surface functionalized latex beads..

MATERIALS

NAME	CATALOG #	VENDOR
500mg Sulfo NHS (N-Hydroxysulfosuccinimide)	BC97	G-Biosciences
EDC N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide	39391-10ML	Sigma – Aldrich

MATERIALS TEXT

- PBS Buffer pH =7.4
- PBS-T Buffer (Tween-20 0.01 %) pH=7.4
- MES Buffer 50 mM pH= 5.9
- Glycine 50 mM aqueous solution
- 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

Reactive Preparations

1 Prepare a 0.4 M aqueous solution of sulfo-NHS. Weight 44.5 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 0.32 M EDC solution. Pipette 29.5 µL of 97% EDC in 470.5 µL of distilled water. EDC solutions must be stored at -20 ºC.

It's convenient to prepare freshly EDC solutions for assuring proper conjugation, unless it will be used within the following days consecutively.

3 Reconstitute the aptamers by pipetting 30 µL of 100 µM stock 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

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

5 **15000 rpm** **4 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).

Latex beads Conjugation

6 Mix on an eppendorf tube 40 µL of sulfo-NHS 0.4 M , 40 µL of 5' amino terminal modified aptamer and 40 µL of 0.32 M EDC solution.

7 Vortex for 20 seconds the prepared 500 µL latex bead suspension in MES and add quickly 160 µL of the conjugation solution prepared in step 6.

8 Cover with aluminium foil or tape the eppendorf tube, and Incubate the prepared solution for 1h 30 minutes at room temperature with strong agitation. (250 rpm on a thermoblock agitator is recommended).

9 Add 100 µL of 50 mM glycine solution. Let the beads incubate for 10 minutes more under agitation. The glycine will react with any of the remaining activated crosslinking groups.

Latex beads Washings

10 **15000 rpm** **4 minutes** the tubeCentrifugate the beads at 15.000 rpm for 4 minutes, discard the supernatant and resuspend the pellet in 500 µL of PBS-T.

Repeat that washing step for three times.

11 Beads can be resuspended in the desired buffer. PBS-T 0.05 % (Tween-20) guarantee adequate beads disperssability in nitrocellulose membranes.

Conjugated beads can be stored at 4ºC for several weeks.