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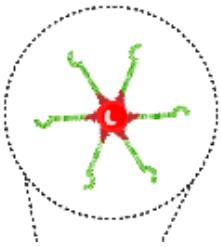
Aptamer 2-step conjugation protocol (INSA-Lyon 2016)



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Protocol status: Other
We have tried and adapted the protocol, the conjugation works properly, however latex nanoparticles tend to aggregate between themselves.

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Abstract

Protocol for bioconjugation of amino terminal modified aptamers with carboxyl surface modified latex beads. Adaptated from iGem team INSA-Lyon 2016.

Guidelines

The following protocol doesn't use sulfo-NHS as an intermedium crosslinking reagent, it reduces significantly the water disperssability of latex beads.

We have not been able to avoid latex beads aggregation during the preparation of aptamer conjugated latex beads following this protocol.

Materials

MATERIALS

Latex beads carboxylate-modified polystyrene fluorescent red 500nm average size. **Catalog #**L3280-1ML

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 + BSA 0.1 %
- MES Buffer 50 mM pH= 5.9
- Amino-terminal modified aptamer, resuspended in distilled water at 6 µM concentration.

Latex Beads Preparation

1 Dilute 480 µL of 2.5 % wt beads stock with 120µL of additional PBS buffer. Reaching a final volume of 500 µL.

2 Centrifugue the tube at 14.000 rpm for 10minutes. Discard the supernatant and resuspend them in 600 µL of MES buffer.

For resuspending beads 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

3 Add quickly 12 mg of EDC (13.7 µL) , and let it stand for 15 min at room temperature.

4 Centrifugate at 14.0000 rpm for 10 minutes, and resuspend in 2 mL of MES buffer. Repeat this step twice.

! We have modified this step, since INSA-Lyon original protocol used PBS for washing and conjugation steps. o-isoacylurea intermediate groups are very inestable , reducing their half life with the pH. At pH higher than 7 they ussualy reacts regenerating carboxyl groups.

5 Aliquot the suspension, adding 500 µL in four different eppendorf tubes.

6 Add 120 µL of amino terminal modified Aptamer (6 µM) to each tube, and incubate them for at least two hours.

Washing and storage

7 Centrifugate the mixture at 14.000 rpm for 10 minutes and wash the pellet twice with PBS + Tween 0.01% w/v and BSA 0.1% w/v. The beads can be stored a few weeks in this buffer. Do not freeze them.