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🌐 Aptamer conjugated beads affinity assay

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We use this protocol and it's working

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ABSTRACT

The following protocol details how to test affinity by an aptamer-conjugated particles with their targets, via target immobilization in 96-treated wells.

GUIDELINES

The steps for coating the wells with the target can differ depending on the molecule to immobilize in the plate.

MATERIALS

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Nunc™ FluoroNunc™/LumiNunc™ 96-Well Plates, clear, C-shaped Bottom,
MaxiSorp, Certified, Clear, For Time-Resolved Fluorescence **Thermo**
Fisher Catalog #437958

- Streptavidin - biotinylated DNA x18 thymine sequence at 8 µM concentration.
- E. Coli suspension in Carbonate-Bicarbonate buffer pH = 9.6 (OD600 = 0.3)
- Conjugated Latex beads Stock at 0.1 % wt in PBS-T 0.05% 1.4 mM MgCl₂
- 5% BSA solution in PBS buffer.
- PBS-T (Tween-20 0.05 %) buffer for washings.

Well Coatings

- 1 Add to each well of the 96 maxisorp plate 100 µL of E. Coli or Streptavidin-DNA suspensions. And incubate overnight at 4 °C.

2 After incubation, remove the liquid in the plate flipping the 96-plate.

! Don't aspire the liquid in the wells with a pipette, to avoid damaging the coating.

3 Add 150 μ L of 3% BSA solution to each well. Make two additional BSA additions for negative controls. Incubate the 96-wells for 4h with mild agitation.

Affinity Assay

4 Add 80 μ L of each conjugated latex beads stock to the coated wells. Remember to add at least in two wells, coated with the different targets respectively; E. Coli and Streptavidin-DNA conjugate. Incubate under mild agitation at room temperature for 1 hour.

5 Remove the liquid by plate flipping, as mentioned in step 2.

6 Wash the wells adding 200 μ L PBS-T (Tween-20 0.05 %), incubating 2 minutes under mild agitation, and flipping the 96-plate. Repeat this step twice.

7 Cover the wells with 50 μ L of distilled water and measure absorbance at the absorption peak for latex beads.

For the the fluorescent orange latex beads we have employed, the absorbance measurement have been performed at 570nm.