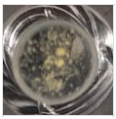

Oct 20, 2019

Aptamer conjugated beads affinity assay

Jorge Fernández[1]

[1]Universidad Complutense de Madrid

1 Works for me dx.doi.org/10.17504/protocols.io.8hxht7n

AEGIS - Madrid iGEM 2019

Jorge Fernandez Mendez
Universidad Complutense de Madrid

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

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

MATERIALS TEXT

- Streptavidin - biotiylated 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 $MgCl_2$
- 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 ºC.

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

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

3 Add 150 µL of 3% BSA solution to each well. Make two adittional BSA adittions for negative controls. Incubate the 96-wells for 4h with mild agitation.

Affinity Assay

4 Add 80 µL 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 200µL 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 µL of distilled water and measure absorbance at the absorbtion peak for latex beads.

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