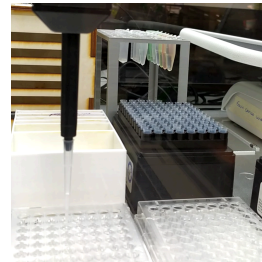


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Cell-ELONA

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Abstract

ELONA (Enzyme-Linked Oligonucleotide Assay), is a biochemical method based on enzyme linked immunosorbent assay (ELISA) that allows to demonstrate that aptamers selected by SELEX can be effective and useful as biorecognition molecules and laboratory tools. The ELONA format choosed uses an anti-digoxigenin antibody to recognize an aptamer previously labelled with digoxigenin. This antibody is conjugated with a peroxidase enzyme, and once it adds ABTS solution, it will be responsible for the colourimetric reaction which will be detected.

(We used 5 replicates per dilution)


Materials

MATERIALS

 MG addgene Catalog #26528

 Anti-Digoxigenin-AP, Fab fragments **Merck MilliporeSigma (Sigma-Aldrich) Catalog #11093274910**


 Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S6014**


 PBST (PBS 1:1000 Tween-20)

 BSA **Merck MilliporeSigma (Sigma-Aldrich)**

 Centrifuge **Eppendorf Catalog #5415D**

 LB **Research Products International Corp (RPI) Catalog #L24400-2000.0**







 ABTS, solution **Thermo Fisher Catalog #002024**

 White 96-Well Immuno Plates, Maxisorp, Flat-Bottom, MaxiSorp, 350µL **Thermo Fisher Catalog #436110**



Before start

Clean all the working surface with ethanol.




Pre-Coating

- 1 Inoculate a single colony of E.Coli DH5 α from LB agar plate in  10 mL of LB. Use a sterile pipette tip, selecting a single colony from LB agar plate. The liquid culture is incubated overnight  37 °C
- 2 Spin at 4000 rpm for  00:05:00 . Discard the supernatant, collect pellet and re-suspend in  10 mL of NaHCO₃-Na₂CO₃, 50mM, pH 9,6. Mix by inverting the tube.
- 3 Spin at 4000 rpm for  00:05:00 . Discard the supernatant, collect pellet and re-suspend in  8 mL of NaHCO₃-Na₂CO₃, 50mM, pH 9,6. Mix by inverting the tube.
- 4 Read the absorbance (600nm). Dilute the sample with NaHCO₃-Na₂CO₃, 50mM, pH 9,6, and adjust the absorbance to 1.










Coating (Automated)

- 5 Dilute the sample 1:4 with NaHCO₃-Na₂CO₃, 50mM, pH 9,6 in a eppendorf tube.
- 6 Add  200 μ L of the bacterial sample into Nunc MaxiSorp 96-well plate and Coating buffer for the negative control. Incubate overnight at  4 °C with agitation (260rpm).

Cell-ELONA (Automated)

- 7 Remove Coating buffer. CAUTION: Be careful to do not touch the well and remove the bacteria.
- 8 Wash 3x  200 μ L with PBS 1x-Tween 0,1%. Remove the drops after the last wash. CAUTION: Be careful to do not touch the well and remove the bacteria.
- 9 Block the plate with  200 μ L PBS 1x BSA 5% for  01:00:00 (260 rpm).



- 10 Structure digoxigenin-labelled aptamers (denatured for 10 min at 95°C and then cooled for 10 min on ice), previously prepared in different concentrations.
- 11 Wash 3x  200 µL with PBS 1x tween 0,1%. Remove the drops after the last wash.
CAUTION: Be careful to do not touch the well and remove the bacteria.
- 12 Add  100 µL /well of the structured aptamers. Incubated for  01:00:00 .
- 13 Wash 3x  200 µL with PBS 1x tween 0,1%. Remove the drops after the last wash.
- 14 Add anti-body antidigoxigenin ( 100 µL /well) preparing 1:1000 dilution in Aptamer buffer-BSA 0,2%. Incubate at room temperature for  01:00:00 .
- 15 Wash 3 x  200 µL with PBS 1x Tween 0,1%.
- 16 Add 100 µL/wall of ABTS solution. Read the absorbance (405 nm) every  00:10:00 for  01:00:00 . ADVICE: We recomend you to buy the ABTS than comes diluted and with the oxygene peroxide.