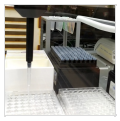

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Cell-ELONA

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1 Works for me dx.doi.org/10.17504/protocols.io.7umhnu6

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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

NAME	CATALOG #	VENDOR
MG	26528	addgene
Anti-Digoxigenin-AP, Fab fragments	11093274910	Sigma – Aldrich
Sodium bicarbonate	S6014	Sigma – Aldrich
PBST (PBS 1:1000 Tween-20)	View	
BSA		Sigma Aldrich
Centrifuge	5415D	Eppendorf Centrifuge
LB	L24400-2000.0	Research Products International (rpi)
ABTS, solution	002024	Thermo Fisher
White 96-Well Immuno Plates, Maxisorp, Flat-Bottom, MaxiSorp, 350µL	436110	Thermo Fisher

BEFORE STARTING

Clean all the working surface with ethanol.

Pre-Coating

1 Inoculate a single colony of E.Coli DH5α 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 $NaHCO_3$-Na_2CO_3, 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 $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6. Mix by inverting the tube.

4 Read the absorbance (600nm). Dilute the sample with $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6, and adjust the absorbance to 1.

Coating (Automated)

5 Dilute the sample 1:4 with $NaHCO_3$-Na_2CO_3, 50mM, pH 9,6 in a eppendorf tube.

6 Add **200 µl** 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 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.

9 Block the plate with **200 µl** 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 concetrations.

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.