Laboratory Protocols

UM_Macau

iGEM_2019

Functional Test

Tyrosinase Activity Assay

1. Homogenize cells (8x10^8 cells) or tissue (50mg) with 500uL ice-cold Tyrosinase Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000xg for 15 minutes at 4 degree.
2. Collect supernatant (lysate) and estimate protein concentration (BCA protein assay kit, protein concentration range from 1-2.5ug/ul. Dilute the lysate if needed using assay buffer)
3. Use the samples for activity analysis immediately, if that is not possible, store them at -80 degree.
4. Prepare 2 wells for each sample: Sample Background Control (SBC) and Sample (S). Add same volume of protein (2-25uL or 5-25ug) into each of these wells.
   For positive control: Add 2 uL of Tyrosinase positive control into the well and add 48uL of Assay Buffer.

For Assay Background Control: Add 50uL Assay Buffer to a well.
For Chromophore Standard Curve Generation:

	Concentration (nmoL/well)	Volume of Chromophore to add (uL)	Volume of Assay buffer to add (uL)
A	0	0	100
B	2	5	95
C	4	10	90
D	6	15	85
E	8	20	80
F	10	25	75

Reaction mix: (Prepare these immediately before adding it to wells)

	SBC mix	Reaction mix
Assay Buffer	45uL	35uL
Substrate	-	10uL
Enhancer	5uL	5uL
	Add SBC mix into SBC wells	Add reaction mix into Assay Background control, Samples and Positive Control wells

5. Mix them well.
6. Have the plate reader ready at 37 degree, at absorbance 510nm on kinetics made set to record absorbance every 30 seconds.
7. Measure at 30 second intervals for 10-15 minutes (for high tyrosinase activity) or for 60-90 minutes (for low tyrosinase activity).

Capture functional test

1. Picked bacteria colony and cultured overnight.
2. Induced 20 mL overnight culture with 1mM IPTG and incubated at 30 C shaking incubator. Centrifuged 10 mL and resuspended with Phosphate buffer. Then measure the OD.
3. Transfer 139 ul of resuspended bacteria samples for both negative and test groups. Put 10 ul of (5000U/mL tyrosinase) to get 333 U/mL (1:15 dilution) into the test groups. Put 10 ul of Phosphate buffer to the negative control.
4. Add 1 ul of 1.0%w/v FP-00552-2 Yellow fluorescent particle, 0.04-0.09 um size in 25C and mixing at 400 rpm for 6 minutes, 12 minutes ,24 minutes or 48 minutes or depending on the experiment objective. Samples were covered with aluminum foil to prevent exposure to light.
5. Centrifuged the solution at 3000xg for 3 minutes to pellet bacteria.
6. Collected supernatant 150ul and put on the 96 well plate. This should contain unbound nanoparticles.
7. Read the fluorescence signal using the filters 440 nm excitation and 480 nm emission.

Setup Diagram:

Test setup 96 well plate format

	1	2	3	4	5	6	7	8	9	10	11	12
A	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1(S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)	OPHT1 (S)(A)
B	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)	OPHT2 (S)(A)
C	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)	OPHT3 (S)(A)
D	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)	OPHM (S)(A)
E	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)	OPHA (S)(A)
F	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
G	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)	+CTRL (Supern atant)
H	6 minutes			12 minutes			24 minutes			48 minutes		

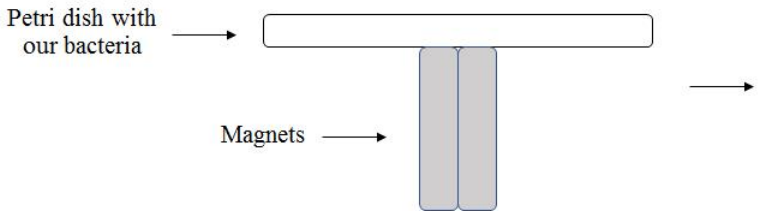
(S) = Supernatant, (P) = Pellet, (IA) = Inactivated, (A)=Activated

Notes for the setup: Each constructs have three technical repeats for each timepoint group as shown in the format above. Supernatant samples were collected and measured the fluorescence amount using a microplate reader.

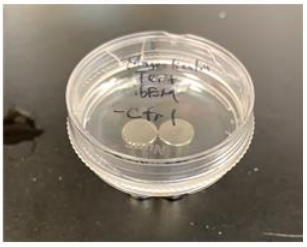
Magnetization assay

1. Pick bacteria colony and culture overnight
2. Induce overnight culture with 0.2% arabinose and continue incubating them at 37 degrees in the shaking incubator for 6-8 hours.
3. Add 20mM ammonium ferric citrate into the bacteria culture and incubate again in 37 degrees for 24 hrs
   **Note: Negative control will not be incubated with ammonium ferric citrate*
4. Centrifuge the bacteria culture and remove the supernatant
5. Resuspend the pellet with autoclaved Milli-Q water
6. Put the resuspended bacteria culture solution onto a petri dish and put the magnet under them as shown in the set-up below:

Setup diagram:



Actual picture of setup:



AgNP and ZnO E.coli survival test

1. Prepared an 5 mL overnight culture of our SANCE E.coli (BL-21 chassis) with ampicillin.
2. Diluted the overnight culture in 1:100 ratio in another 5 mL LB broth with ampicillin.
3. Prepared 5 different concentration of Zinc Oxide and Silver nanoparticles in LB broth solution. 0.5 mg/L, 1 mg/L, 20 mg/L, 40 mg/L and 60 mg/L.
4. Put 200 ul of LB broth with ampicillin in the blank groups.
5. Put 100 ul of LB broth with the Zinc Oxide and Silver Nanoparticle groups respectively.
6. Put 100 ul of the 1:100 bacteria LB Broth in the treatment.
7. Read initial OD600 absorbance values as initial time.
8. Incubated in 37 C shaking incubator and read absorbance values for 2 hrs, 4 hrs, 6 hrs, 8 hrs and 12 hrs. This is to check the cell viability after being exposed to toxic nanoparticles.

Setup Diagram

96 well plate format							
		ZnO			AgNP		
		1	2	3	4	5	6
Blank	A	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
0 mg/L	B	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
0.25 mg/L	C	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
0.5 mg/L	D	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
10 mg/L	E	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
20 mg/L	F	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
30 mg/L	G	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
	H						