Three enzymatic activities were measured

Saturday , April 14th , 2023

Overview:

- Experiments were performed by measuring the activities of ADH, FALDH, and FDH by using a UV-spectrophotometer

Experimental equipment:

- And 10 mM methanol, 10 mM formaldehyde, and 10 mM formic acid
- 1mM NAD+
- 10 mM enzyme (A2, M8, T7)

Experimental steps:

① experimental operation procedures

- The operation step is a 300ul measurement system with a final concentration of 1 mM A 2
- First, 30ul NAD +, 30ul 10mM of methanol, and 210ul buffer were then added in a 1.5-ml EP tube
- The solution was passed through the pipette professionally to a cuvette and zero (find the AUTO-ZERO button on the computer)
- After zero, 30ul A2 was added to the cuvette. Quickly press the "measure" button on the computer to start the measurement
- The measurement could be stopped by 2min-3min of the reaction

② experimental result

- Changes of Abs in each group over time under different experimental conditions

detection result

	A	B	C	D	E	F	G	H	I	J	K
1		1mM methanol 30 Protein	And 1.5 mM of methanol 30 Protein	Of 1.5 mM methanol, and 30 Protein, 1.5mM N AD +	0.75 mM Methanol 30 protein	1mM formaldehyde 30 Protein	0.75 mM formaldehyd e 30 protein	0.5 mM formaldehyde 30 Protein	And 1.5 mM formic acid 30 Protein	1mM formic acid 60 Protein	1mM formic acid 30 Protein
2	0s	0.004	0.056	0	0.02	0.012	0.053	0.041	0.017	0.086	-0.002
3	30s	-0.004	0.049	-0.036	0.005	0.011	0.097	0.093	0.017	0.02	0.003
4	60s	-0.003	0.05	-0.032	0.002	0.015	0. 137	0. 1 15	0.014	0.014	0
5	90s	-0.001	0.052	-0.027	0.006	0.019	0. 159	0. 129	0.009	0.016	0.001
6	120s	0	0.051		0.015	0.023	0. 174	0. 139	-0.021	0.022	

- Conclusion: It can be seen from the above data that A2 has the highest activity under 1.5 mM methanol and 30 protein; T7 has the highest activity under 0.75 mM formaldehyde and 30 protein; and M8 performs poorly under three conditions

Transformation of M8

Sunday, April 16th, 2023

Overview

- The PET28A-Mko-CL8 (M8) was transformed into BL21 competent cells

Experimental equipment

- Plasmid: PET28A-Mko-CL8 (M8)
- The competent cell, BL21
- LK culture medium
- NZY, culture medium

Experimental steps

- 1 μL of plasmid was mixed with 30 μL of competent cells
- Ice bath for 30min

- Heat shock: put in a 42℃ water bath, 45S
- Ice bath 2min
- 150 μL of NZY medium was added to the superclean workbench
- Shaker 37℃ 40 to 60 min
- Apply the LK medium plates
- 37 ℃ incubator

Pick bacteria

Monday , April 17th, 2023

Overview

- Positive transformed single colonies grown on lk plates were inoculated and grown in lk liquid medium

Experimental equipment

- Transformed E. coli, liquid lk medium

Experimental steps

- Pour one-third of the lk medium into the small culture vial
- Positive transformed single colonies grown on plates were picked
- The picked colonies were inoculated in lk liquid medium
- Culture in a 37-degree shaker

Preparation of large bottles and SDS-PAGE gel

Tuesday April 18th, 2023

Overview

- Connect the bacteria from the bottle into the large bottle

Experimental equipment

- Phosphate Buffer (4)
- Kanamycin (2000)
- TB culture medium
- bacteria solution

Experimental steps

① Experimental procedure (feeding)

- Put all the required items into the ultra-clean workbench (except for bacterial liquid) and shine with UV for 10min
- 25ml of 4 phosphate buffer, 2000 kanamycin 500 ml and 2~3ml solution were added to TB
- The TB medium of the bacteria was incubated in a constant temperature aker at 37 degrees Celsius for about 10h

② Preparation of the SDS-PAG E glue

Add the corresponding reagents according to the following table

Tabl e 1

	A	B
1	Reagent name	Dose (ml)
2	Lower glue (15 ml 12% Gel)	
3	H 2O	4.9
4	30%Acrylamide	6.0
5	.51MTris -HCK (pH 8.8)	3.8
6	10%SDS	0.15
7	And 10% ammonium persulfate	0.15
8	TEMED	0.006

9	Upper adhesive (10ml)	
10	Reagent name	Dose (ml)
11	H 2O	6.8
12	30%Acrylamide	1.7
13	.01MTris -HCK (pH 6.8)	1.25
14	And 10% ammonium persulfate	0.1
15	TEMED	0.01

Bacteria and prepared for protein purification

Wednesday , April 19th, 2023

Overview

- The solution in TB medium was successively centrifuged to obtain the desired bacteria

Experimental equipment

- revulsive
- centrifuge
- receiving flask
- centrifuge tube
- The ultrafiltration tube

Experimental steps

① receiving bacteria

- The inducer was added to the already treated TB medium and induced at 18 ° C
- Remove TB culture medium, put into the collection bottle and flattened in pairs, and removed in a centrifuge for 10min at 6000 r/min for 10 min
- The supernatant was poured, and buffer (20 ml pbs-buffer + 380m water l) was washed and transferred to a centrifuge tube
- The centrifuge tube was flattened in pairs and put into a centrifuge and centrifuged

for 20min at 4,000 r/min 5 ° C and removed

② Prepare for protein purification

- Clean the ultrafiltration tube

Breaking bacteria, protein purification, and electrophoresis

T hursday , April 20th , 2023

Overview

- The bacteria cultured in TB were broken, and the target proteins were obtained by affinity chromatography, and finally the results were analyzed by glue running.
- at different pH was measured together

Experimental equipment

- Breaking bacteria: autoclaved bacterial breaker, PBS buffer, pmfs
- Protein purification: Nickel beads, PBS buffer, pmfs, imidazole, G250 Coomassie brilliant blue
- SDS-PAGE: matched SDS-PAGE glue, loading, protein marker

Experimental steps

① broken bacteria

- Bacteria were weighed on a balance and 1g was added to 10ml PBS buffer and resuspended
- Add pmsf (protease inhibitor) to the resuspended solution, and 10ml to 100ul pmsf
- Wash the filter 2 times with clean water first, then 2 times with buffer (and the pressure is kept at 300bar~450bar during cleaning)

- After washing, the bacterial liquid was added to the instrument for crushing, and the breaking pressure was kept at 800bar~900bar
- Repeated break 4~5 times, so that the bacterial liquid is no longer sticky
- After breaking, 2mlEP tubes and then centrifuged 14000 rpm 30min 10℃.
- All supernatants and little precipitation can be collected.

② protein was purified

- Add some nickel beads to the purified container, wash them twice with water and then twice with buffer
- The nickel beads were then activated with 10 mM imidazole (10 mM imidazole in buffer configuration, pour in and stand for 3~5min)
- The supernatant was added to the protein-purified container and sealed with a sealing membrane. Place in the mute mixer for not less than 1h
- In a 50ml centrifuge tube, 1 / 1000 pmfs was added and marked
- 100ul G250 of Coomassie brilliant blue was added to each well in the microplate
- The nickel bead protein was eluted with 10 mM imidazole, and 10ul was added to G250 to see if it turned blue (blue represents protein containing)
- Repeat the previous step until the G250 is unchanged blue
- Increase the imidazole concentration and repeat steps 13,14
- Mark each sample well

③ electrophoresis

- Take 4 loading 10ul + 40ul sample (drain, supernatant, precipitate, imidazole eluate at each concentration)

- Cook 100℃ for 10min, then centrifuge slightly
- Sample and protein marker were added to the matched SDS-PAGE gel (10ul for sample and 2ul for marker)
- Protein glue needs constant current run (30 mA voltage per plate glue can be increased), about 40~60min
- After stained with dye (for hours)
- Decoloring is done after staining (overnight decoloring)

Transformation (A2、M8)

Friday, April 21st, 2023

Overview

- The PET28A-Mko-CL8 (M8) and PET28a-ADH-CL2 was transformed into BL21 competent cells respectively

Experimental equipment

- Plasmid: PET28A-Mko-CL8 (M8)
- The competent cell, BL21
- LK culture medium
- NZY, culture medium

Experimental steps

- 1 μL of plasmid was mixed with 30 μ L of competent cells
- Ice bath for 30min
- Heat shock: put in a 42℃ water bath, 45S
- Ice bath 2min
- 150 μ L of NZY medium was added to the superclean workbench
- Shaker 37℃ 40 to 60 min
- Apply the LK medium plates
- 37 ℃ incubator

A 2, M 8 protein purification and electrophoresis

Tuesday, April 25th, 2023

Overview

- The protein solution was washed with different concentrations of imidazole and washed with G250 dye. The washed protein liquid was prepared and analyzed by electrophoresis staining.

Experimental equipment

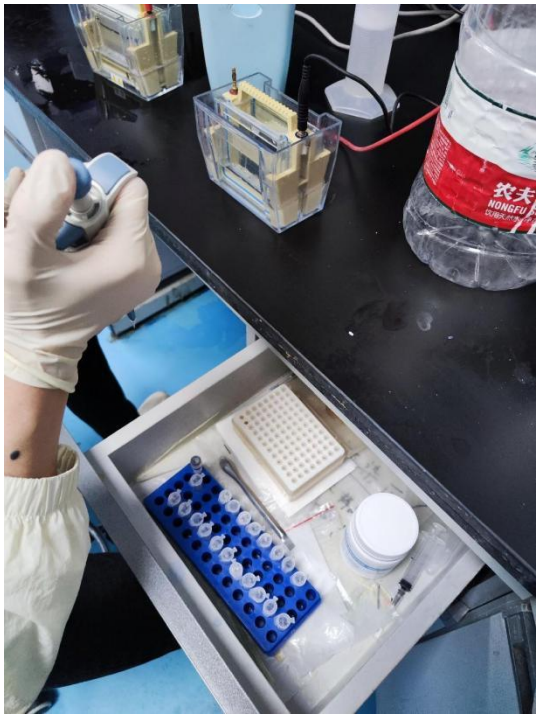
- A2, M8 protein solution
- Pipette gun, gun head, 50ml centrifuge tube, 10ml tubing, small row hole
- Different concentrations of imidazole (103050100150300) mM
- PMSF protease inhibitor
- Protein glue, loading, and protein manufacturer

Experimental steps

① purification

- Use 50ml centrifuge tube, add PMSF and mark
- Was eluted with 10 mM imidazole,
- For ot, add PMSF to each eluate; change a higher concentration of imidazole
- Repeat the above procedure until 300 mM imidazole
- Mark each elution tube and place it in ice

② sample preparation and electrophoresis



- 20ulload + 80ul sample {supernatant, fluid, [10,30,50,100 (1) 100 (2) 150 (1) 150 (2) 300] mM imidazole eluate}
- Mark each sample tube
- Heat in a 100℃ metal bath for 10min and slightly centrifuged
- Add sample and maker to the prepared protein glue (protein sample plus 10ul, maker plus 2ul)
- Set the current, one plate glue 30 mA, run 40-60min
- After running, the rubber plate was stained and placed in a horizontal shaking for several hours
- The next day it was decigmented and analyzed

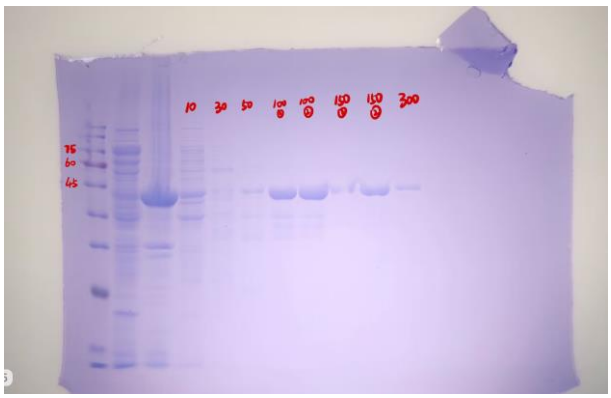


Figure 1: Results of gel electrophoresis(A2) electrophoresis(M8)

Figure 2: Results of gel

protein ultrafiltration (A2、M8)

Wednesday, April 26th, 2023

Overview

- The purified protein was raised to its concentration by ultrafiltration

Experimental equipment

- 1× pbs buffer
- The G250 Coomassie Bright Blue

Experimental steps

- After washing the ultrafiltration centrifuge tubes, they were filled with RO water and added to 4M NaOH 1ml for 15min to inactivate the residual protein
- After soaking, pour RO water containing NaOH, wash with RO water, and soak with RO water
- When use, pour RO water, and add RO water to the scale line in the upper container. After leak detection at 4000 rpm 10℃ 1min in the centrifuge, the corresponding buffer is added to the centrifuge for 4000 rpm 10℃ 1min
- After leak detection, the target protein solution was added for 4000 rpm 10℃ 10min, 10ul of upper and lower liquid was taken, and tested by G250 (upper blue and lower constant blue)
- After G250 test, pour the lower liquid, fill the target protein liquid to the

scale line and centrifuge, replace the upper liquid to the scale line with buffer (twice) and leave 500ul-1500ul of protein liquid to be divided (after 200ul of 1.5ml EP tube, quick-frozen with liquid nitrogen and stored at-80℃)

Concentration and activity of the three enzymes

Thursday, April 27th, 2023

Overview

- This experiment determined the concentration and activity of the two enzymes of A2 and M8 and T7 obtained in the previous experiments

Experimental equipment

- Unknown concentration of methanol dehydrogenase, formaldehyde dehydrogenase, and formate dehydrogenase
- 1 mM NAD +, 10 mM methanol, 10 mM formaldehyde, 100 mM sodium formate, 0.2M Na2HPO4 buffer

Experimental steps

①Concentrations were measured by

- Wash the wells of nucleic acid instrument with water and EB solution, and absorb the water with the wells and EB solution with suction paper
- The plasmid solution of 2-4 ul was then successively up with a pipette gun and placed into the wells
- Cover the instrument lid, debug the equipment, and click Test

- The concentrations of the different plasmids were recorded and analyzed, and then photographed

② measured the activity

- Open the software and set the address of the measurement parameters and the saved data
- First, 15ul 1M NAD +, 15ul 10mM methanol / formaldehyde and appropriate amount of ulpH solution were added to 1.5ml EP tubes, including the negative control with only ph solution and enzyme
- The above solution was transferred to a pivette and zeroing out (find the AUTO-ZERO button on the computer)
- After zero, 50 ul of A 2 / F7 protein solution was added to the cuvette. Quickly press the "measure" button on the computer to start the measurement

Experimental result

- According to the experimental data mentioned above, the concentration of A2 is 3.027 mg/ml, the concentration of T7 is 2.850 mg/ml, and the concentration of M8 is 3.197mg / ml. A2 and M8 enzymes showed unsatisfactory activity, with T7 still most active at 0.75 mM formaldehyde and 30ulT