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Entry 5/5: Assay after 8.4
In Project: iGEM TUK 2020 Labjournal
No tags associated

created: 27.10.2020 18:31
updated: 27.10.2020 21:05

8/6/2020-*E. coli* BaLac BL21 Freezing ABTS Assay

T. versicolor								
2	200 total volume=4microl 200dilution+196microl buffer							<0. 04mM
200	200 total volume=20microl stock enzyme(pH7)+180microl buffer (dialysis)							

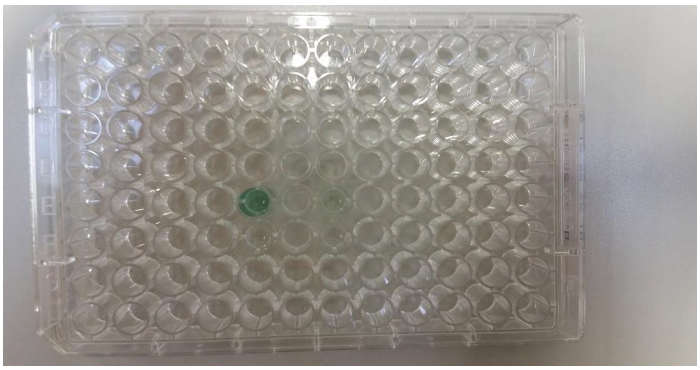
ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer

	BaLac obtained:		
	110.67 microM for 500microl	Control Enzyme:T. versicolor	Purified Enz BaLac
	Well	5	6
	Enzyme Concentration	0.04mM	0.006mM
	pH		

a	4		
b	4		
c	4		
d	4		
e	4	5µl TVenzy dilution +5µlABTS+190µl buffer	195 µl 1enzyme+5 0µl buffer
f	Control 4	5µl TVenzy dilution +0µlABTS+195µl buffer	195 µl 1enzyme+0 5µl buffer
g	4		
h	4		
			**Buffer wi substituted dialysis buf in this assay
			Odd white c samples. Nc this is enzyr

Data_Set_08.06_BaLac_frozen.xlsx

8/6 BarLac plate.png



8/13/2020-*T. versicolor* Positive Control ABTS Assay, Diclofenac Incubation Spectra

	Tversicolor
2	200 total volume=2microl 200dilution+198microl buffer (pH5)
200	200 total volume=20microl stock enzyme(pH7)+180microl buffer(pH7)
	Same dilution will be used for both salt and normal buffer, salt will just be added in place of buffer

ABTS Dilution prep			
ABTS	300µl	30µl ABTS	270µlpH5buffer

		Control Enzyme:T. versicolor	
	Well	5	6
	Enzyme Concentration	0.04mM	3.57mM
	pH		
a	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
b	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
c	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
d	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
e	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
f	5	5µl 2enzy dilution +10µlABTS+185µl buffer	5µl 200enzy dilut +10µlABTS+185µ
g	5+Buffer-ABTS	5µl 2enzy dilution +0µlABTS+195µl buffer	5µl 200enzy dilut +0µlABTS+195µl
h	5-enzyme		0 µl enzyme+10µl buffer

Diclofenac Incubation Spectra

Diclofenac
dilution
Needs to be 20microg/ml (optimal according to paper is this, ABTS is 0.25mM, however at this concentration it would be 80microg/ml, we should be able to detect
lower)

0.25mM from
ABTS
Diclofenac MW 318.13g
/mol
per 1ml=79.
5325microg

First make 20mg dilution in H2O to prevent inaccurate measurements
20mg/1ml

>>20mg=20000microg

Needs 1microl of dilution per 999microl pH buffer (4,5,7)

HOWEVER because we're using a 1:1 of diclo/laccase, we need to double the
concentration

2microl diclofenac dilution (20mg/ml)+998microl buffer (each pH)

>>Due to solubility for diclo only being 0.004mg/ml, 20mg were added to 5ml making 4mg/ml
conc
Then 10microl diclo dilution was added to each pH
990microl

Final probe prep		
	Control	Incubation
pH5	100microl pH5 buffer+ 100microl pH5 40 diclo	100microl pH5 200 laccase+ 100microl pH5 40 diclo
	100microl pH5 buffer	
	100microl pH 200 laccase+ 100microl pH buffer	
	>>Take 100microl of each and measure in spectro after 10 min incubation	
	EACH FINAL WELL WILL HAVE 20MICROG/ML (~0.0625mM?) DICLO AND 100MICROG/ML (1.79mM) LACCASE CONCENTRATIONS	
	***We should calculate diclo's concentration from same we did for ABTS	

pH_5_Day1.xlsx

pH_5_Day2.xlsx

pH4&pH7.xlsx

pH5_HPLC_Heat.xlsx

pH5_HPLC_Heat_customized.xlsx

8/19/2020-*E. coli* BaLac BL21 ABTS Assay

	BaLac obtained:	47,24µM for ~900µL -> Seperated them to a volumen of 450µL for each , one tested today, one frozen	
		Control Enzyme:T. versicolor	Purified Enzyme: BaLac
	Well	5	6
	Enzyme Concentration	200µg/mL	0.047mM
a	pH		
b	4		
c	4	5µl T-enzy dilution +5µlABTS+190µl buffer	195 µl B-enzyme+5µlABTS+0 µl buffer
d	4	5µl T-enzy dilution +5µlABTS+190µl buffer	
e	Control 4	5µl T-enzy dilution +0µlABTS+195µl buffer	195 µl B-enzyme+0µlABTS+5 µl buffer

Data_Set_08.19_BaLac.xlsx

8/19 BarLac plate.png

8/24/2020-*E. coli* BaLac BL21 Freezing ABTS Assay

ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer

New Mastermix
160 microl ABTS buffer + 6080 microl buffer

		Same as at 19.8. but with frozen enzyme BaLac	
		Control Enzyme:T. versicolor	Purified Enzyme: BaLac
	Well	5	6
	Enzyme Concentration	200µg/mL	0.047mM
a	pH		
b	4		
c	4	5µl T-enzy dilution +5µlABTS+190µl buffer	
d	4	5µl T-enzy dilution +5µlABTS+190µl buffer	195 µl B-enzyme+5µlABTS+0 µl buffer
e	Control 4	5µl T-enzy dilution +0µlABTS+195µl buffer	195 µl B-enzyme+0µlABTS+5 µl buffer

Data_Set_08.24_BaLac_frozen.xlsx

8/26/2020-*E. coli* marLac ABTS Assay

ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer

New Mastermix
160 microl ABTS buffer + 6080 microl buffer

Obtained: MarLac 71.56 µM in 1.5mL		Control Enzyme:T. versicolor	Purified Enzyme: MarLac
	Well	5	6
	Enzyme Concentration	200µg/mL	0.071mM
a	pH		
b	7		195 µl B-enzyme+5µlABTS+0 µl buffer
c	7		195 µl B-enzyme+5µlABTS+0 µl buffer
d	7		195 µl B-enzyme+5µlABTS+0 µl buffer

e	7		195 µl B-enzyme+5µlABTS+0 µl buffer
f	7	5µl T-enzy dilution +5µlABTS+190µl buffer	195 µl B-enzyme+5µlABTS+0 µl buffer
g	7	5µl T-enzy dilution +5µlABTS+190µl buffer	195 µl B-enzyme+5µlABTS+0 µl buffer
h	Control 4	5µl T-enzy dilution +0µlABTS+195µl buffer	195 µl B-enzyme+0µlABTS+5 µl buffer

Data_Set_08.26_MarLac.xlsx

8/31/2020-*T. versicolor* Positive Control ABTS Assay

ABTS Dilution prep					
ABTS	200µl	20µl ABTS	180µlpH4buffer	then:	160 microl ABTS buffer + 6080 microl buffer

used dialysis buffer from e.coli group (pH4 and pH7)

T. versicolor	
200	200 total volume=20microl stock enzyme(pH7)+180microl buffer (dialysis)

Obtained: MarLac 71.56 µM in 1.5mL		Control Enzyme: T. versicolor	Purified Enzyme: MarLac	Enzy
	Well	5	6	7
	Enzyme Concentration	200µg/mL	0.071mM	0 en
a	pH			
b	7		195 µl B-enzyme+5µlABTS+0 µl buffer	
c	7		195 µl B-enzyme+5µlABTS+0 µl buffer	
d	7		195 µl B-enzyme+5µlABTS+0 µl buffer	

e	7		195 µl B-enzyme+5µlABTS+0 µl buffer	
f	7	5µl T-enzy dilution +5µlABTS+190µ l buffer	195 µl B-enzyme+5µlABTS+0 µl buffer	0 µl enzy µl bu
g	7	5µl T-enzy dilution +5µlABTS+190µ l buffer	195 µl B-enzyme+5µlABTS+0 µl buffer	0 µl enzy µl bu
h	Control 4	5µl T-enzy dilution +0µlABTS+195µ l buffer	195 µl B-enzyme+0µlABTS+5 µl buffer	0 µl enzy µl bu
	Well	5	6	7
	Enzyme Concentration	no ABTS	200	no e
a				
b				
c	dialysis buffer pH 4	5µl 200enzy µg /ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 +5µl

d	dialysis buffer pH 4	5µl 200enzy µg /ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 +5µl
e				
f	dialysis buffer pH 7	5µl 200enzy µg /ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 +5µl
g	dialysis buffer pH 7	5µl 200enzy µg /ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 +5µl

Data_Set_08.31.xlsx

9/3/2020-Prepare new stocks, *T. versicolor* Positive Control ABTS Assay

Preperation of new T. versicolor stock
Used 20mg of T.versicolor and mixed it with 1mL of UF water, stored in the fridge

T. versicolor	
200	200 total volume=20microl stock enzyme(pH7) +180microl buffer(dialysis)

ABTS Dilution prep				
ABTS	200µl	20µl ABTS	180µlpH4buffer	then:
used dialysis buffer from e.coli group (pH4 and pH7)				

	Well	5	6	
	Enzyme Concentration	no ABTS	200	no enzyn
a				
b				
c	dialysis buffer pH 4	5µl 200enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 µl Bu buffer
d	dialysis buffer pH 4	5µl 200enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 µl Bu buffer
e				
f	dialysis buffer pH 7	5µl 200enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 µl Bu buffer
g	dialysis buffer pH 7	5µl 200enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	100 µl Bu buffer

Data_Set_09.03.xlsx

9/7/2020-Prepare new stocks, *T. versicolor* Positive Control ABTS Assay

Stocks:			(Want/have) * volume
T. versicolor	20mg/ml		
ABTS	100mM		
Enzyme calc			
Want	200microg/ml	=	0.2mg/ml
Have	20mg/ml		
Volume	200microl		
((0.2mg/ml)/(20mg/ml)) * 200microl		=	2microl enzyme
ABTS calc			
Want	0.25mM		
Have	100mM		Each well
Volume	200microl		
(0.25mM/100mM) * 200microl		=	0.5microl ABTS

	Well	5	
	Enzyme Concentration	200microg/ml	No enzyme
a			
b		pH4	
c	pH 4	2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	0µl 200enzy µg/ml +0.5µlABTS +1 5µl buffer
d	pH 4	2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	
e	pH 4	2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	

Data_Set_09.07..xlsx

9/8/2020-*T. versicolor* Positive Control ABTS Assay

	Well	5	
	Enzyme Concentration	200microg/ml	No enzyme
a			
b		pH4	
c		2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	0µl 200enzy µg/ml +0.5µlAB 5µl buffer
d		2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	
e		2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer	

Data_Set_09.08..xlsx

9/9/2020-*E. coli* BaLac BL21 ABTS Assay

T. versicolor positive control			
2microg/ml	=	0.04mM	
Enzyme calc			
Want	2microg/ml	=	0.002mg/ml
Have	20mg/ml		
Volume	200microl		
((0.002mg/ml)/(20mg/ml)) * 200microl		=	0.02microl enzyme
ABTS calc			
Want	0.25mM		
Have	100mM		Each well
Volume	200microl		
(0.25mM/100mM) * 200microl		=	0.5microl ABTS

	BaLac obtained:	
	31.106microM for about 350microl	Control Enzyme:T. versicolor
	Well	
	Enzyme Concentration	0.04mM=postive control
	pH	
a	4	
b	4	
c	4	
d	4	2µl 200enzy µg/ml +0.5µlABTS +197.5µl buffer>200microg/ml
e	4	0.02µl 2enzy µg/ml +0.5µlABTS +199.48µl buff
f	Control 4	0.02µl 2enzy µg/ml +0.5µlABTS +199.98µl buff
g		
h		
		Adding a 200microg/ml just in case for positive control

2020.09.09.xlsx

9/28/2020-Chlamy Lysate BaLac ABTS Assay

ABTS Dilution prep			
ABTS	20µl	2µl ABTS	18µlpH4
ABTS	20µl	2µl ABTS	18µlpH5
ABTS	20µl	2µl ABTS	18µlbuffer

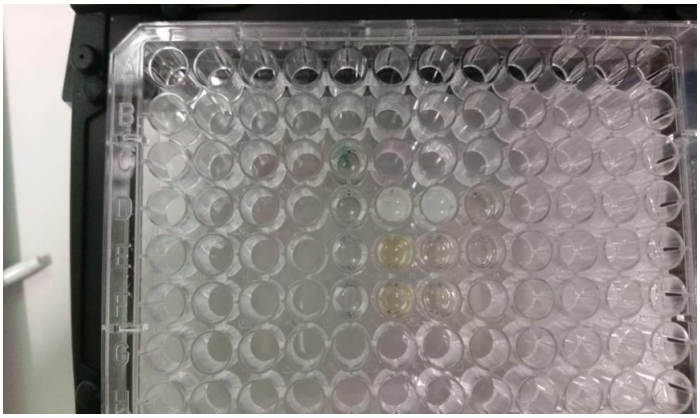
T. versicolor	
200	20 total volume=2microl stock enzyme (pH7)+18microl buffer(pH5)

EDTA/TRIS Buffer pH=7.5
BaLac optimal pH=4
TV optimal ph=5

	BaLac obtained:	
	136.47?microM for 500microl	
	This is likely mixed with other proteins	Control Enzyme:T. versicolor
	Well	
	Enzyme Concentration	Pos control
	pH	
a		
b		
c	Optimal pH	5µl TVenzy dilution +5µlABTS+190µl pH5
d	Half optimal pH/half buffer	5µl TVenzy dilution +5µlABTS+90µl pH5+ 85µlbuffer
e	Buffer	5µl TVenzy dilution +5µlABTS+190µl buffer
f		5µl TVenzy dilution +0µlABTS+195µl buffer

29.09_igem.xlsx

9/29 Chlamy plate.png



10/5/2020-Chlamy Lysate BaLac ABTS Assay

10/12/2020-Chlamy Lysate BaLac ABTS Assay

ABTS Dilution prep			
ABTS	40µl	4µl ABTS	36µlpH6.5
ABTS	40µl	4µl ABTS	36µlpH5

T. versicolor	
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH6.5)
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH5)

EDTA/TRIS Buffer pH=7.5
BaLac optimal pH=4
TV optimal ph=5

	BaLac obtained:	
	136.47?microM for 500microl	
	This is likely mixed with other proteins	Control Enzyme:T. versicolor
	Well	5
	Enzyme Concentration	Pos control
	pH	
a	pH6.5	
b	pH5	
c	pH6.5	5µl TVenzy dilution +5µlABTS+190µl buffer
d	pH5	5µl TVenzy dilution +5µlABTS+190µl buffer
e	pH6.5	5µl TVenzy dilution +0µlABTS+195µl buffer
f	pH5	5µl TVenzy dilution +0µlABTS+195µl buffer

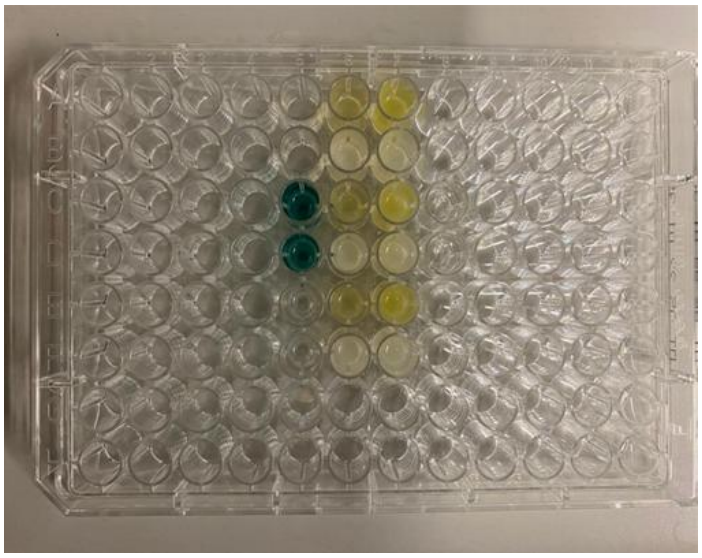
T. versicolor	
200	20 total volume=2microl stock enzyme (pH7)+18microl buffer(pH6.4)
2	200 total volume=2microl 200dilution+198microl buffer (pH6.4)
200	20 total volume=2microl stock enzyme (pH7)+18microl buffer(pH5)
2	200 total volume=2microl 200dilution+198microl buffer (pH5)

	BaLac obtained:		
	0.2microg/ml or less	Control Enzyme:T. versicolor	Purified Enzyme:Ba
	Well	5	
	Enzyme Concentration	Pos control	Sample
	pH for sample		
a	pH6.4 With Copper	5µl TVenzy 200 dilution +5µlABTS+190µl bufferpH6.4	195 µl 1enzyme+5µ Copper
b	pH5 With Copper	5µl TVenzy 2 dilution +5µlABTS+190µl bufferpH6.4	195 µl 1enzyme+5µ
c	pH6.4 Without Copper	5µl TVenzy 200 dilution +5µlABTS+190µl bufferpH5	195 µl 1enzyme+5µ Copper
d	pH5 Without Copper	5µl TVenzy 2 dilution +5µlABTS+190µl bufferpH5	195 µl 1enzyme+5µ Copper
e	pH6.4 With Copper	5µl TVenzy 2 dilution +0µlABTS+195µl bufferpH6.4	195 µl 1enzyme+0µ Copper
f	pH5 With Copper	5µl TVenzy 2 dilution +0µlABTS+195µl bufferph5	195 µl 1enzyme+0µ
g	pH6.4 Without Copper		195 µl 1enzyme+0µ Copper
h	pH5 Without Copper		195 µl 1enzyme+0µ Copper

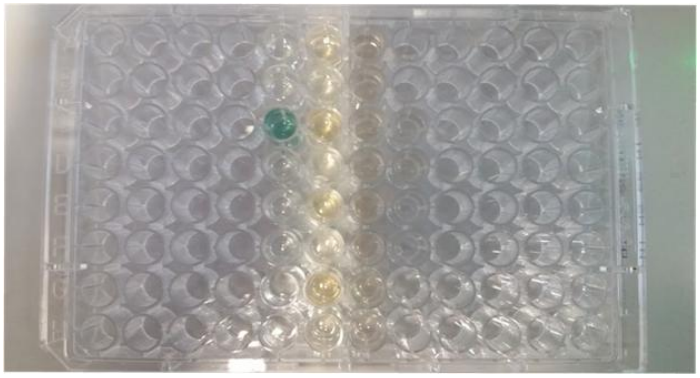
05_10_2020.xlsx

chlamy_14.10.xlsx

10/5 Chlamy plate.png



10/12 Chlamy plate.png



10/14/2020-HPLC with *T. versicolor* and *E. coli* BaLac BL21

10/15/2020-HPLC, Chlamy Lysate BaLac Cu Comparison ABTS Assay

Run Diclofenac at 3 different concentrations			
First make stocks:			pH4 Buffer
Want	Working stock		
80microg/ml	160microg/ml		40microl Stock4mg/ml + 960microl buffer
20microg/ml	40microg/ml	>>>	10microl Stock4mg/ml + 990microl buffer
10microg/ml	20microg/ml	>>>	5microl Stock4mg/ml + 995microl buffer
5microg/ml	10microg/ml	>>>	2.5microl Stock4mg/ml + 997.5microl buffer

Add 100 microl of each working concentration to 100 microl buffer to create wanted concentration.
Test 50microl of sample into 250microl phosphoric acid buffer
Test 50microl of pH4 into 250microl phosphoric acid buffer to see background noise
After optimal concentration is determined, add this to T. versicolor dilution 100microg/ml

T. versicolor							
200	400 total volume=40microl stock enzyme(pH7)+360microl buffer(pH4)						Wanted concentration 100microg/ml
2	400 total volume=4microl 200dilution+396microl buffer (pH4)						Wanted concentration 1microg/ml

	Start time for TV 100microg/ml +80microg/ml diclo:	Starttime for 1microg/ml TV + 80microg/ml diclo		BaLac 200microl of 7microM	
T0	16.33	16.43		t0	1925
T30	17 03	17 13		T10	1935
T60	17 33	17 43		T30	1955
T120		1843		T60	2025
T10	1907			T120	2125
50microl of BaLac+50microl of 80microg/ml diclo					

	HPLC Results:	Area for 13.1 peak			
	TV100	TV1	BaLac 7microM	Other	
t0	559.87939	101.26219	824.94281		
t10	64.20737	27.33621	145.40282		
t30	193.84665		176.68774		*balac t30 had a peak at 13.4 at this area
t60	38.58089	99.68481	225.40498		
Diclo10				113.99874	10
Diclo40				869.25244	21
Diclo80				2636.37012	32
KiltTV100+Diclo80				25.99939	
Buffer				0	

HPLC_Results.pptx

hplc.xlsx

hplc2.xlsx

10/15/2020-HPLC, Chlamy Lysate BaLac Cu Comparison ABTS Assay continued

ABTS Dilution prep			
ABTS	40µl	4µl ABTS	36µlpH6.5
ABTS	40µl	4µl ABTS	36µlpH5

T. versicolor	
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH6.4)
2	200 total volume=2microl 200dilution+198microl buffer (pH6.4)
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH5)
2	200 total volume=2microl 200dilution+198microl buffer (pH5)

BaLac optimal pH=4
TV optimal ph=5
Step down pH6.4

	BaLac obtained:								

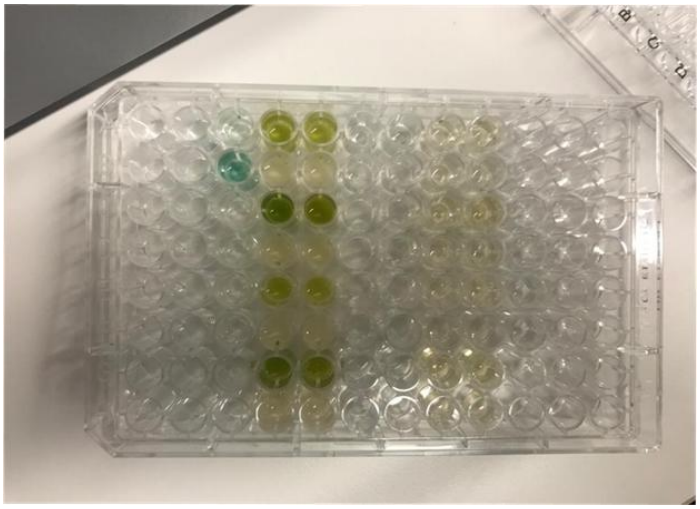
	7microg/ml (all samples diluted to this level)	Control Enzyme: T. versicolor	Purified Enzyme: BaLac	Purified Enzyme: BaLac	Purified Enzyme: BaLac	Enzyme control	Enzyme control	Enzyme control	No enzyme
	Well	3	4	5			8	9	10
	Enzyme Concentration	Pos control 3570microM (200) or 40microM (2)	Sample1 7microM	Sample2 7microM			Neg enzyme WILD2 7microM	Neg enzyme WILD3 7microM	Neg control 0microM
	pH for sample								
a	pH6.4 With Copper	5µl TVenzy 200 dilution +5µlABTS+190µl bufferpH 6.4	195 µl 1enzyme +5µlABTS+0µl buffer pH6.4 With Copper	195 µl 1enzyme +5µlABTS+0µl buffer pH6.4 With Copper			195 µl Wenzyme+5µlABTS+0µl bufferpH6.4 With Copper	195 µl Wenzyme+5µlABTS+0µl bufferpH6.4 With Copper	
b	pH5 With Copper	5µl TVenzy 200 dilution +5µlABTS+190µl bufferpH5	195 µl 1enzyme +5µlABTS+0µl bufferpH5 With Copper	195 µl 1enzyme +5µlABTS+0µl bufferpH5 With Copper			195 µl Wenzyme+5µlABTS+0µl bufferpH5 With Copper	195 µl Wenzyme+5µlABTS+0µl bufferpH5 With Copper	

c	pH6.4 Without Copper	5µl TVenzy 2 dilution +5µlABTS+190µl bufferpH 6.4	195 µl 1enzyme +5µlABTS+0µl bufferpH 6.4 Without Copper	195 µl 1enzyme +5µlABTS+0µl bufferpH6 .4 Without Copper			195 µl Wenzym e+5µlABTS+0µl bufferpH6 .4 Without Copper	195 µl Wenzym e+5µlABTS+0µl bufferpH6 .4 Without Copper	
d	pH5 Without Copper	5µl TVenzy 2 dilution +5µlABTS+190µl bufferpH5	195 µl 1enzyme +5µlABTS+0µl bufferpH 5 Without Copper	195 µl 1enzyme +5µlABTS+0µl bufferpH5 Without Copper			195 µl Wenzym e+5µlABTS+0µl bufferpH5 Without Copper	195 µl Wenzym e+5µlABTS+0µl bufferpH5 Without Copper	
e	pH6.4 With Copper	5µl TVenzy 2 dilution +0µlABTS+195µl bufferpH 6.4	195 µl 1enzyme +0µlABTS+5µl bufferpH 6.4 With Copper	195 µl 1enzyme +0µlABTS+5µl bufferpH6 .4 With Copper			195 µl Wenzym e+0µlABTS+5µl bufferpH6 .4 With Copper	195 µl Wenzym e+0µlABTS+5µl bufferpH6 .4 With Copper	0 µl enzyme+ 5µlABTS +195µl bufferpH6 .4
f	pH5 With Copper	5µl TVenzy 2 dilution +0µlABTS+195µl bufferph5	195 µl 1enzyme +0µlABTS+5µl bufferpH 5With Copper	195 µl 1enzyme +0µlABTS+5µl bufferpH5 With Copper			195 µl Wenzym e+0µlABTS+5µl buffer pH5With Copper	195 µl Wenzym e+0µlABTS+5µl buffer pH5With Copper	0 µl enzyme+ 5µlABTS +195µl bufferphH5

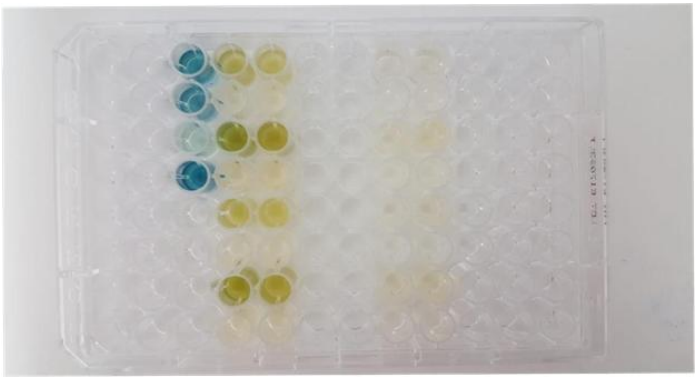
g	pH6.4 Without Copper		195 µl 1enzyme +0µlABTS+5µl buffer pH6.4 Without Copper	195 µl 1enzyme +0µlABTS+5µl buffer pH6.4 Without Copper			195 µl Wenzyme+0µlABTS+5µl buffer pH6. 4Without Copper	195 µl Wenzyme+0µlABTS+5µl buffer pH6. 4Without Copper	
h	pH5 Without Copper		195 µl 1enzyme +0µlABTS+5µl buffer pH5 Without Copper	195 µl 1enzyme +0µlABTS+5µl buffer pH5 Without Copper			195 µl Wenzyme+0µlABTS+5µl buffer pH5Without Copper	195 µl Wenzyme+0µlABTS+5µl buffer pH5Without Copper	

15102020_Balac_intra.xlsx

10/15 chlamy 1.png



10/15 Chlamy2.png



10/7/2020-*E. coli* marLac, BaLac AD494, and BaLac Rosetta ABTS Assay

ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH7buffer
ABTS	200µl	20µl ABTS	180µlpH4buffer

T. versicolor	
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer (pH7)
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer (pH4)
2	200 total volume=2microl 200dilution+198microl buffer (pH4)

Obtained: MarLac 23.85µM in 200microL			
Obtained: AD494 BaLac 26.8 µM in 300microL		Control Enzyme:T. versicolor	Pu
	Well	5	
	Enzyme Concentration	3570microM (200) or 40microM (2)	23
a	pH		
b			
c			
d		5µl 2-enzy dilution +5µlABTS+190µl buffer pH4	19 pH
e		5µl 200-enzy dilution +5µlABTS+190µl buffer pH4	
f		5µl 200-enzy dilution +5µlABTS+190µl buffer pH7	
g		5µl 200-enzy dilution +0µlABTS+195µl buffer pH7	

ABTS Dilution prep		
ABTS	200µl	20µl ABTS

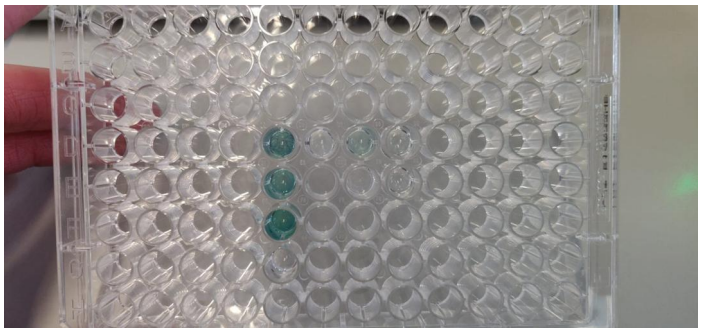
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH4)
2	200 total volume=2microl 200dilution+198microl buffer (pH4)

Obtained: Rosetta gami BaLac 27.5 µM in 200microL		Control Enzyme:T. versicolor	Purified Enzyme:BaL
	Well	5	
	Enzyme Concentration	200µg/mL	27.5mM
a	pH		
b	4		
c	4		
d	4		
e	4		
f	4	5µl 2-enzy dilution +5µlABTS+190µl buffer pH4	195 µl B-enzyme+5µ pH4
g	4	5µl 200-enzy dilution +5µlABTS+190µl buffer pH4	
h	4	5µl 200-enzy dilution +0µlABTS+195µl buffer pH4	

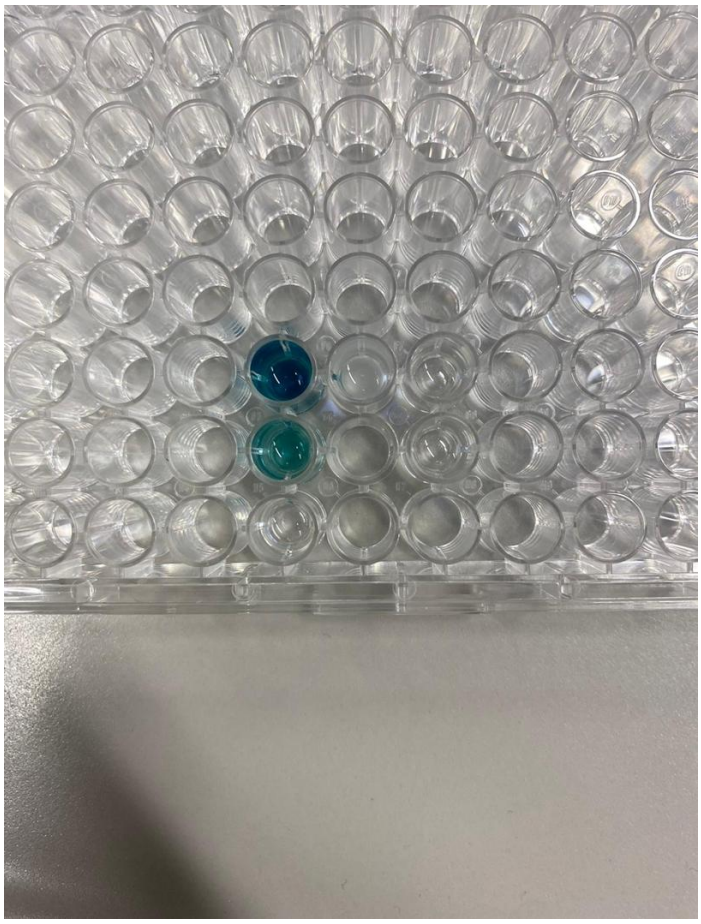
BarLac_Rosetta_0910.xlsx

Assay_BL21mar_AD494Ba_20201007_(1).xlsx

10/7 marLac and BaLac AD494.png



BaLac Rosetta gami.png



10/21/2020-*E. coli* BaLac AD494 ABTS Assay, HPLC Spectra

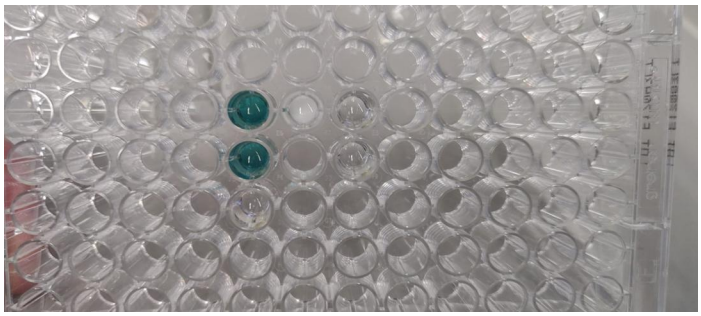
ABTS Dilution prep		
ABTS	200µl	20µl ABTS

T. versicolor	
200	20 total volume=2microl stock enzyme(pH7)+18microl buffer(pH4)
2	200 total volume=2microl 200dilution+198microl buffer (pH4)

	Obtained: BaLac 37.41µM in 200microL	Control Enzyme:T. versicolor	Purifie
	Well	5	
	Enzyme Concentration	200µg/mL	37.4mi
a	pH		
b	4		
c	4		
d	4	5µl 2-enzy dilution +5µlABTS+190µl buffer pH4	195 µl buffer p
e	4	5µl 200-enzy dilution +5µlABTS+190µl buffer pH4	
f	4	5µl 200-enzy dilution +0µlABTS+195µl buffer pH4	

Assay_AD494Ba_2020.10.22.xlsx

10/21 BaLac AD494.png



Summary.xlsx

All_assays.pptx