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Entry 4/4: Assay Protocol
In Project: iGEM TUK 2020 Labjournal
No tags associated

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Lab Journal

Table of Contents

5/25/2020-Made Buffers

Stock Solutions				Notes
	Buffers			
	Basic Solution			
	Company	Carl Roth GmbH+Co.	Na_2HPO_4	Di-natriumhydrogen phosphat-dihydrat
		Formular Weight: 177.99g/mol		Calculation
	Calculated	44.4975g per 250ml		177.99g/mol*0.25=44.4975g per 250ml
	Actual	44.5g	Added powder to 250ml bottle	
		Making buffer with ultra filtrated H_20		
		Fill 250ml bottle to 200ml with UF H_20		
		Moved this mixture to 1 L container		

		Added H_20 to 1L creating 0.25 molar concentration		
	Acidic Solution			
	Company	Sigma	Citric Acid	trisodium salt dihydrate
		Folmular Weight: 294.1g/mol		Calculation
	Calculated	For 250ml solution: 73.525g		294.1g/mol*0.25=73.525g in 250ml
	Calculated	For 100ml solution: 29.41g		73.525g(100ml/250ml)=29.41g
	Actual	29.42g		
		Filled 250ml bottle with 29.42g of powder and willed to 50ml with UF H_20		
		Stirred/shook till clear then filled to 100ml		
		Created 1 molar solution		
		Citric acid monohydrate		
		Formular weight: 210,14g/mol		210,14g/mol*0,25=52,535g in 250ml

After recalculating to avoid second dilution, this is corrected stock solution amounts:

	Na2HPO4	NEED		
	177,99 g/mol	0,2 M	200 ml	
	CALCULATION: 177,99 g /mol * 0,2 M * 0,2 l = 7,119 g			
	Citric acid	NEED		
	210,14 g/mol	0,1 M	200 ml	
	CALCULATION: 210,14 g /mol * 0,1 M * 0,2 l = 4,203 g			
	pH 7: 164,7 ml Na2HPO4 + 35,3 ml Citric acid			
		For 200 ml of Buffer		
	pH 4: 77,1 ml Na2HPO4 + 122,9 ml Citric acid			

5/26/2020-Buffer Dilutions

Calculations						
Enzyme		0.5u/mg in bottle		1u=1µmol/min	1µmol=0.1µ ABTS	10m incubation
	10u/ml	=	20mg /ml			
	0.5u/mg			c=1mM ABTS	mM=mmol/L	

				c=n/v	v=1ml=0.001L	
				1µmol ABTS (n) in 1 ml (v)		
				1µmol/1ml		
				1u/10m=0.1u=need 1/5mg		
Citric Acid Buffer						
	0.1 M want	(100ml)	10ml buffer	or for 200ml	20ml buffer	
	1M have		90ml H_20		180ml H_20	
Basic Buffer	0.2 M Want	(100ml)	80ml buffer	or for 200ml	160ml buffer	
	0.25 M Have		20ml H_20		40ml H_20	
Prepared	Basic Buffer	0.2M				
	and					
	Citric Acid Buffer	0.1M				
	with above buffers we made 3 different pH buffers as follows					
	pH want	0.2M Na_2HPO_4	Citric Acid	Actual pH	Final pH*	
	4	7.71ml	12.29ml	8,51	3,95	
	5	10.3ml	9.7ml	9	5,04	
	7	16.47ml	3.53ml	9,28	7,04	
	*This is the final pH after adjustment using conc. Hydrochloric acid at approximately 32% and 10M NaOH					

ABTS		$C_{18}H_{16}N_4O_6S_4$-$(NH_4)_2$-Mr548.7				
	c=n/v	54,87	=	0.05487g/ml		
		1000				
		548.7g=1mol				
		54.87 g=100mmol				
Actual	enzyme	0.278mg in 1.39 ml 7.0 pH solution			v=m/c	
	Claculated ABTS:	actual weighted /c				
		0.0584g/0.0547g	=	1.068ml UF H_20		

5/27/20-ABTS abs spectra with *T. veriscolor* at different pH's

ABTS:	1mU	(1ml)	=	0.01 ml	=	10 uL	
	100mU						
Enzyme:	0.2mg/ml	=	1ml	=	0.01 ml	=	10uL
	20mg/ml						
Buffer:	total volume	=	980uL				
	Filled 9 eppendorf tubes with:						
	pH 4 buffer 980uL	10uL UF H_20	10uL ABTS				
	pH 4 buffer 980uL	10uL UF H_20	10uL Enzyme				

	pH 4 buffer 980uL	10uL ABTS	10uL Enzyme				
	pH 5 buffer 980uL	10uL UF H_20	10uL ABTS				
	pH 5 buffer 980uL	10uL UF H_20	10uL Enzyme				
	pH 5 buffer 980uL	10uL ABTS	10uL Enzyme				
	pH 7 buffer 980uL	10uL UF H_20	10uL ABTS				
	pH 7 buffer 980uL	10uL UF H_20	10uL Enzyme				
	pH 7 buffer 980uL	10uL ABTS	10uL Enzyme				
	UV-Visible System Spectrometer-Chem Station						
	Using pH 7 buffer to blank for enzyme						
	See folders /documents on Allyssa's flashdrive for data						
Time point	1	pH 4 buffer with ABTS					
	2	pH 4 buffer with ABTS and enzyme					
	3	pH 4 buffer with ABTS and enzyme			2m		
	4	pH 4 buffer with ABTS and enzyme			4m		

	5	pH 4 buffer with ABTS and enzyme		8m		
	6	pH 4 buffer with ABTS and enzyme		10m		
	7	pH 4 buffer with ABTS and enzyme		12m		
	8	pH 4 buffer with ABTS and enzyme		15m		
Time point	1	pH 5 buffer with ABTS				
	2	pH 5 buffer with ABTS and enzyme				
	3	pH 5 buffer with ABTS and enzyme		2m		
	4	pH 5 buffer with ABTS and enzyme		4m		
	5	pH 5 buffer with ABTS and enzyme		6m		
	6	pH 5 buffer with ABTS and enzyme		8m		
	7	pH 5 buffer with ABTS and enzyme		10m		
	8	pH 5 buffer with ABTS and enzyme		12m		
Time point	1	pH 7 buffer with ABTS				
	2	pH 7 buffer with ABTS and enzyme				
	3	pH 7 buffer with ABTS and enzyme		2m		

	4	pH 7 buffer with ABTS and enzyme			4m		
	5	pH 7 buffer with ABTS and enzyme			6m		
	6	pH 7 buffer with ABTS and enzyme			8m		
	7	pH 7 buffer with ABTS and enzyme			10m		
	8	pH 7 buffer with ABTS and enzyme			12m		
	9	pH 7 buffer with ABTS and enzyme			13m		
	*For future experiments maybe lower enzyme concentration						

5-27_pH4_data.xlsx

5-27_pH5.xlsx

5-27_pH7.xlsx

6/2/2020-*T. versicolor* Positive Control ABTS Assay

pH	Concentrations Enyzme ug per ml												
4	0	2	10	20	50	100	200	ABTS		pH4 ABTS	200µl ABTS	20µl ABTS	180µl pH4 buffer
4	0	2	10	20	50	100	200	ABTS		Enzyme	200µl Enzyme	20µl Enzyme	180µl buffer

control	0	2	10	20	50	100	200	ABTS pH4control		pH5			
5	0	2	10	20	50	100	200	ABTS		ABTS	200µl ABTS	20µl	180µl pH5 buffer
5	0	2	10	20	50	100	200	ABTS		Enzyme	200µl Enzyme	20µl	180µl buffer
control	0	2	10	20	50	100	200	ABTS pH5control		pH7			
7	0	2	10	20	50	100	200	ABTS		ABTS	200µl ABTS	20µl	180µl pH7 buffer
7	0	2	10	20	50	100	200	ABTS		Enzyme	200µl Enzyme	20µl	180µl buffer
control= enzyme without ABTS							14 controls without ABTS *pH4 and 5						
							8 controls without Enzyme in all pH						
0.5µl Conc value µg/ml enyzme+0.5µlABTS+199µl buffer						Can't pipette 0.5microl, need to adjust dilutions with buffer and increase to 5microl each							
5µl Conc value µg/ml enyzme+5µlABTS+190µl buffer													

Concentration calculations

Dilution amounts		
Concentration enzyme	µl enzyme/previous dilution	Notes
2	100 total volume=20microl 20 enzyme+80microl buffer	
10	100 total volume=50microl 20 enzyme+50microl buffer	
20	200 total volume=80microl 50 enzyme+120microl buffer	*final volume 130microl after r
50	200 total volume=100microl 100 enzyme+100microl buffer	*final volume 120microl after r
100	200 total volume=100microl 200 enzyme+100microl buffer	*final volume 100microl after r
200	200 total volume=20microl stock enzyme+180microl buffer	*final volume 100microl after r

6/3/2020-*T. versicolor* Positive Control ABTS Assay

T. versicolor prep

Enzyme dilution prep			
pH 4		pH 5	
2	100 total volume=20microl 20 enzyme(pH4)+80microl buffer(pH4)	2	100 total volume=2 (pH5)
10	100 total volume=50microl 20 enzyme(pH4)+50microl buffer(pH4)	10	100 total volume=5 (pH5)
20	200 total volume=80microl 50 enzyme(pH4)+120microl buffer(pH4)	20	200 total volume=8 (pH5)
50	200 total volume=100microl 100 enzyme(pH4)+100microl buffer (pH4)	50	200 total volume=1 (pH5)
100	200 total volume=100microl 200 enzyme(pH4)+100microl buffer (pH4)	100	200 total volume=1 (pH5)
200	200 total volume=20microl stock enzyme (pH7)+180microl buffer (pH4)	200	200 total volume=2 (pH5)

ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer
ABTS	200µl	20µl ABTS	180µlpH5buffer
ABTS	200µl	20µl ABTS	180µlpH7buffer

96 well plate set up:

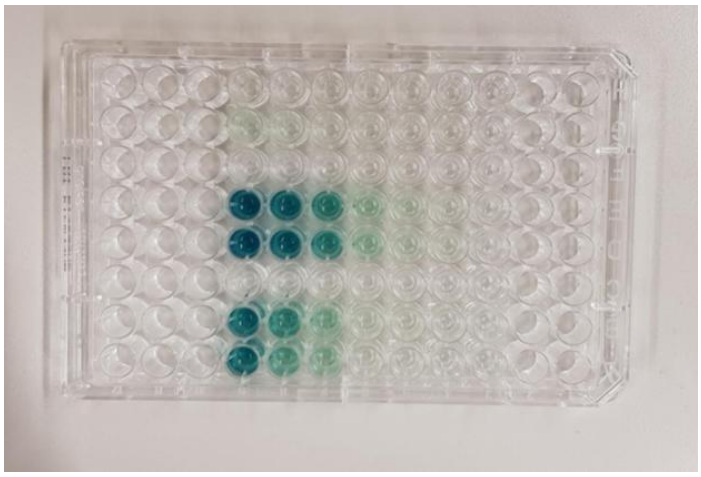
Well	3	4	5	6	7	8	9	
Enzyme Concentration	0	2	10	20	50	100	200	
pH	Concentrations Enyzme ug per ml							
4	5µlABTS +195µl buffer	5µl 2enzy µg/ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	ABTS
4	5µlABTS +195µl buffer	5µl 2enzy µg/ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	ABTS

control	195µl buffer+5ul buffer	5µl 2enzy µg/ml +195µl buffer	5µl 10enzy µg/ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	ABTS pH 4control
5	5µlABTS +195µl buffer	5µl 2enzy µg/ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	ABTS
5	5µlABTS +195µl buffer	5µl 2enzy µg/ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	ABTS
control	195ul buffer+5ul buffer	5µl 2enzy µg/ml +195µl buffer	5µl 10enzy µg/ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	ABTS pH 5control
7	5µlABTS +195µl buffer	5µl 2enzy µg/ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	ABTS

7	5µlABTS +195µl buffer	5µl 2enzy µg /ml +5µlABTS+ 190µl buffer	5µl 10enzy µg/ml +5µlABTS+ 190µl buffer	5µl 20enzy µg/ml +5µlABTS+ 190µl buffer	5µl 50enzy µg/ml +5µlABTS+ 190µl buffer	5µl 100enzy µg/ml +5µlABTS+ 190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	A B TS
5µl Conc value µg /ml enyzme+ 5µl ABTS+ 190 µl buffer		*pH5 abts buffer mix for b4						
					Premix ABTS and buffer in future assays			

6-3_TV_assay.xlsx

Plate 6/3



6/8/2020-*T. versicolor* Positive Control ABTS Assay

Enzyme dilution prep			
pH 4		pH 5	
2	100 total volume=20microl 20 enzyme(pH4)+80microl buffer (pH4)	2	100 total volume=20microl 20 en
10	100 total volume=50microl 20 enzyme(pH4)+50microl buffer (pH4)	10	100 total volume=50microl 20 en
20	200 total volume=80microl 50 enzyme(pH4)+120microl buffer(pH4)	20	200 total volume=80microl 50 en
50	200 total volume=100microl 100 enzyme(pH4)+100microl buffer (pH4)	50	200 total volume=100microl 100
100	200 total volume=100microl 200 enzyme(pH4)+100microl buffer (pH4)	100	200 total volume=100microl 200
200	200 total volume=20microl stock enzyme (pH7)+180microl buffer (pH4)	200	200 total volume=20microl stock

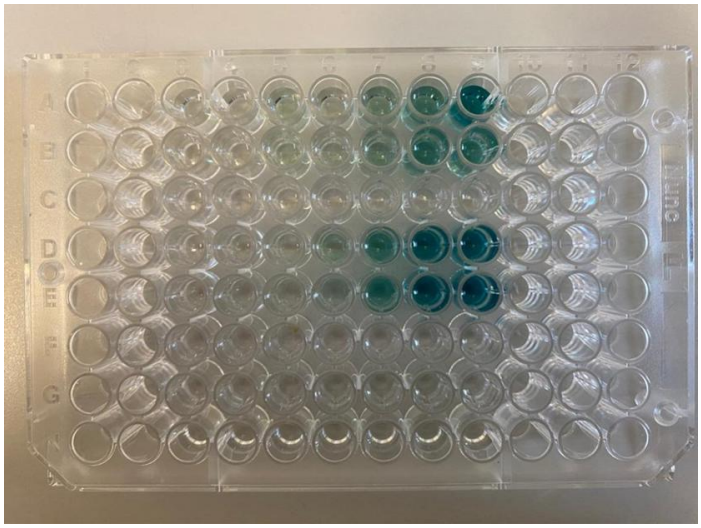
ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer
ABTS	200µl	20µl ABTS	180µlpH5buffer
ABTS	200µl	20µl ABTS	180µlpH7buffer

Calculations for mastermix ABTS/Buffer (need one for each pH)		
32x190 Buffer (each pH)	pH4	pH5
32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 6080 microl buffer	160 microl ABTS buffer + 6(

	Well	3	4	5	6	7
	Enzyme Concentration	0	2	10	20	50
	pH	Concentrations Enyzme ug per ml				
a	4	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer
b	4	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer
c	control	195µl buffer+5ul buffer	5µl 2enzy µg/ml +195µl buffer	5µl 10enzy µg/ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 50e +195µl
d	5	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer
e	5	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer
f	control	195ul buffer+5ul buffer	5µl 2enzy µg/ml +195µl buffer	5µl 10enzy µg/ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 50e +195µl
g	7	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer
h	7	5µlABTS+195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50e +5µlAB buffer

6-8_TV_assay.xlsx

6/8 plate



6/15/2020-Preparation of Solutions

Preparing more 4/5/7 buffers			40 ml total	
Prepared	Basic Buffer	0.2M		
	and			
	Citric Acid Buffer	0.1M		
	with above buffers we made 3 different pH buffers as follows			
	pH want	0.2M Na_2HPO_4	Citric Acid	Actual
	4	15.42ml	24.58ml	8..960
	5	20.6ml	19.4ml	9..100
	7	32.94ml	7.06ml	9..220
	*This is the final pH after adjustment using conc. Hydrochloric acid at approximately 32% and 10M NaOH			
Prepared				
BLUE tubes for ABTS/Buff mix	Calculations for mastermix ABTS/Buffer (need one for each pH)			
	32x95 Buffer (each pH)	pH4	pH5	pH7
	32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 3040 microl buffer	160 microl ABTS buffer + 3040 microl buffer	160 mi
Prepared				
Actual				
	ABTS	0.0548g/0.0548g	=	1.00ml
		0.548g/0.0547g	=	ml UF

6/16/2020-Chlamy ABTS Assay cancelled due to miscommunication

6/18/2020-Chlamy Lysate ABTS Assay

Go from 20-200microg/ml, increase time for test to 2 hours

Enzyme dilution prep			
pH 4		pH 5	
20	200 total volume=80microl 50 enzyme(pH5)+120microl buffer(pH4)	20	200 total volume=80m
50	400 total volume=200microl 100 enzyme(pH4)+200microl buffer (pH4)	50	400 total volume=200
100	400 total volume=200microl 200 enzyme(pH4)+200microl buffer (pH4)	100	400 total volume=200
200	400 total volume=40microl stock enzyme (pH7)+360microl buffer (pH4)	200	400 total volume=40m

ABTS Dilution prep			
ABTS	200µl	20µl ABTS	180µlpH4buffer
ABTS	200µl	20µl ABTS	180µlpH5buffer
ABTS	200µl	20µl ABTS	180µlpH7buffer

	Add 100 microl of mastermix to each well, add 100 of each dilution to each well.	
BLUE tubes for ABTS/Buff mix	Calculations for mastermix ABTS buffer/Buffer pH (need one for each pH)	
	32 wellsx95 microl Buffer (each pH)	pH4
	32 wellsx5 microl ABTS (each buffer dilution)	160 microl ABTS buffer + 3040

		Replicate 1					Replicate 2			
	Well	3	4	5	6	7	8	9	10	11

	Enzyme Concentration	20	50	100	200	0	20	50	100	200
	pH					Concentrations Enyzme ug per ml				
a	4	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95 µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer

b	4	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer
c	control	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	100 µl Buffer +5µl ABTS+95µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer

d	5	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer
e	5	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer

f	control	5µl 20enzy µg/ml +195 µl buffer	5µl 50enzy µg/ml +195 µl buffer	5µl 100enzy µg/ml +195 µl buffer	5µl 200enzy µg/ml +195 µl buffer	100 µl Buffer +5µl ABTS+95 µl buffer	5µl 20enzy µg/ml +195 µl buffer	5µl 50enzy µg/ml +195 µl buffer	5µl 100enzy µg/ml +195 µl buffer	5µl 200enzy µg/ml +195 µl buffer
g	7	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95 µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer

h	7	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer	100 µl Buffer +5µl ABTS+95µl buffer	5µl 20enzy µg/ml +5µl ABTS+190µl buffer	5µl 50enzy µg/ml +5µl ABTS+190µl buffer	5µl 100enzy µg/ml +5µl ABTS+190µl buffer	5µl 200enzy µg/ml +5µl ABTS+190µl buffer

b8-b11 mixed in just 5microl abts, not abts buffer
e8-e11 mixed in just 5microl abts, not abts buffer
h8-h11 mixed in just 5microl abts, not abts buffer

6-18_Chlamy_assay.xlsx

6/22/2020-*T. versicolor* Positive Control ABTS Assay

Go from 20-200microg/ml, increase time for test to 4 hours

Step 1				
	ABTS Dilution prep			
	ABTS	200µl	20µl ABTS	180µlpH4buffer
	ABTS	200µl	20µl ABTS	180µlpH5buffer
	ABTS	200µl	20µl ABTS	180µlpH7buffer

OPTIONAL STEP 2	>>>Otherwise just mix ABTS and Buffer in wells separately	
BLACK mix		
Calculations for mastermix ABTS/Buffer (need one for each pH)		
32x190 Buffer (each pH)	pH4	pH5
32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 6080 microl buffer	160 microl ABTS buffer + 6

	Step 3: Fill in main wells with ABTS /Buffer for each					
	Replicate 1					Repli 2
Well	3	4	5	6	7	8

Enzyme Concentration	20	50	100	200	0	20
pH					Concentrations Enyzme ug per ml	
4	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe
4	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe

control	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	195µl buffer+5ul buffer	5µl 20enz /ml +195µ buffe
5	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe
5	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe
control	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	195ul buffer+5ul buffer	5µl 20enz /ml +195µ buffe

7	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe
7	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS +190µl buffer	5µl 100enzy µg/ml +5µlABTS +190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20enz /ml +5µlA +190µ buffe

Step 4: Mix Enzyme dilutions	Enzyme dilution prep		
pH 4		pH 5	
20	200 total volume=80microl 50 enzyme(pH5)+120microl buffer(pH4)	20	200 total volume=80m
50	400 total volume=200microl 100 enzyme(pH4)+200microl buffer (pH4)	50	400 total volume=200
100	400 total volume=200microl 200 enzyme(pH4)+200microl buffer (pH4)	100	400 total volume=200
200	400 total volume=40microl stock enzyme (pH7)+360microl buffer (pH4)	200	400 total volume=40m

Step 5: In second well plate, add around 10microl of each enzyme dilution, then when assay begins, add enzyme to plate and insert into machine.

6-22_TV_assay.xlsx

6/23/2020-Prepare new stocks, *T. versicolor* Positive Control ABTS Assay

MONOHYDRATE!						
Citric Acid Buffer	210.14g/mol molecular weight		210.14g/mol x 0.25 L volume		>> 52.535g /250ml	
	0.1 M want	(100ml)	10ml buffer	or for 200ml	20ml buffer	
	1M have		90ml H_20		180ml H_20	
					created 1M solution	
Prepared	Basic Buffer	0.2M				
	and					
	Citric Acid Buffer	0.1M				
	with above buffers we made 3 different pH buffers as follows					
	pH want	0.2M Na_2HPO_4	0.1MCitric Acid	Actual pH	Final pH*	
	4	7.71ml	12.29ml	3..90		
	5	10.3ml	9.7ml	4..92		
	7	16.47ml	3.53ml	7..04		
	*This is the final pH after adjustment using conc. Hydrochloric acid at approximately 32% and 10M NaOH					
ABTS Stock						
		0.0546g/0.0547g	=	0.998ml UF H20		

Preparation of Assay

Step 1				
	ABTS Dilution prep			
	ABTS	200µl	20µl ABTS	180µlpH4buffer
	ABTS	200µl	20µl ABTS	180µlpH5buffer
	ABTS	200µl	20µl ABTS	180µlpH7buffer

OPTIONAL STEP 2	>>>Otherwise just mix ABTS and Buffer in wells separately	
BLACK mix		
Calculations for mastermix ABTS/Buffer (need one for each pH)		
32x190 Buffer (each pH)	pH4	pH5
32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 6080 microl buffer	160 microl ABTS buffer + 6

		Step 3: Fill in main wells with ABTS /Buffer for each			***ROW SIX SKIPPED FOR ENZYME, ROW 7 USED!!!		
		Replicate 1					Rep 2
	Well	3	4	5	6	7	8

	Enzyme Concentration	20	50	100	200	0	20
	pH					Concentrations Enyzme ug per ml	
a	4	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buf
b	4	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buf

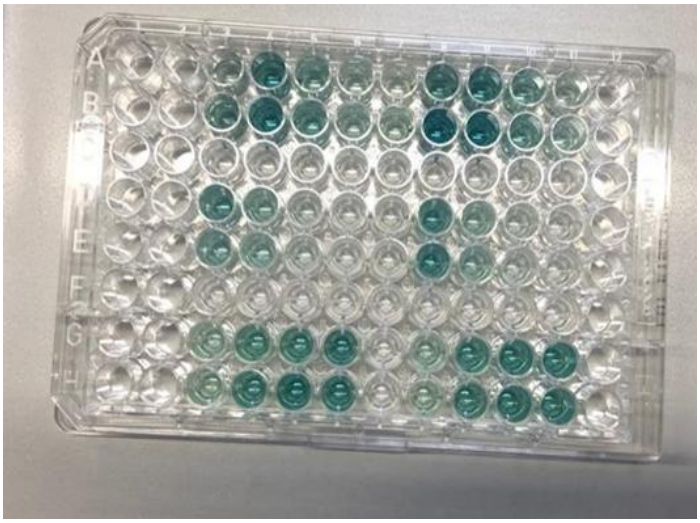
c	control	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg /ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	195µl buffer+5 ul buffer	5µl 20e /ml +19 buff
d	5	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg /ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buff
e	5	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg /ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buff
f	control	5µl 20enzy µg/ml +195µl buffer	5µl 50enzy µg /ml +195µl buffer	5µl 100enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +195µl buffer	195ul buffer+5 ul buffer	5µl 20e /ml +19 buff

g	7	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buf
h	7	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µl 50enzy µg/ml +5µlABTS+190µl buffer	5µl 100enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl 20e /ml +5µ S+1 buf

Enzyme dilution prep			Step 4: Mix Enzyme dilutions		
pH 4		pH 5		pH 7	Double the amount
20	200 total volume=80microl 50 enzyme(pH5)+120microl buffer (pH4)	20	200 total volume=80microl 50 enzyme(pH5)+120microl buffer (pH4)	20	200 total volume=80microl 50 enzyme(pH5)+120microl buffer (pH4)
50	400 total volume=200microl 100 enzyme(pH4)+200microl buffer (pH4)	50	400 total volume=200microl 100 enzyme(pH4)+200microl buffer (pH4)	50	400 total volume=200microl 100 enzyme(pH4)+200microl buffer (pH4)
100	400 total volume=200microl 200 enzyme(pH4)+200microl buffer (pH4)	100	400 total volume=200microl 200 enzyme(pH4)+200microl buffer (pH4)	100	400 total volume=200microl 200 enzyme(pH4)+200microl buffer (pH4)
200	400 total volume=40microl stock enzyme (pH7)+360microl buffer (pH4)	200	400 total volume=40microl stock enzyme (pH7)+360microl buffer (pH4)	200	400 total volume=40microl stock enzyme (pH7)+360microl buffer (pH4)

Step 5: In second well plate, add around 10microl of each enzyme dilution, then when assay begins, add enzyme to plate and insert into machine.

6-23 plate



6/29/2020-*T. versicolor* Positive Control ABTS Assay

ABTS Stock calculation			
	0.0547g/0.0548g	=	0.998ml UF H20

Go from 2-20mg/ml, increase time for test to 4 hours

Step 1	ABTS Dilution prep			
	ABTS	200µl	20µl ABTS	180µlpH4buffer
	ABTS	200µl	20µl ABTS	180µlpH5buffer
	ABTS	200µl	20µl ABTS	180µlpH7buffer

OPTIONAL STEP 2	>>>Otherwise just mix ABTS and Buffer in wells separately	
BLACK mix		
Calculations for mastermix ABTS/Buffer (need one for each pH)		
32x190 Buffer (each pH)	pH4	pH5
32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 6080 microl buffer	160 microl ABTS buffer + 6

		Step 3: Fill in main wells with ABTS /Buffer for each					
		Replicate 1					Rep 2
	Well	3	4	5	6	7	8

	Enzyme Concentration	2	10	15	20	0	2
	pH					Concentrations Enyzme ug per ml	
a	4	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ml +5µ S+1 buff
b	4	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ml +5µ S+1 buff

c	control	5µl 2enzy µg /ml +195µl buffer	5µl 10enzy µg /ml +195µl buffer	5µl 15enzy µg /ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	195µl buffer+5 ul buffer	5µl µg/ +19 buff
d	5	5µl 2enzy µg /ml +5µlABTS+190µl buffer	5µl 10enzy µg /ml +5µlABTS+190µl buffer	5µl 15enzy µg /ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ +5µ S+1 buff
e	5	5µl 2enzy µg /ml +5µlABTS+190µl buffer	5µl 10enzy µg /ml +5µlABTS+190µl buffer	5µl 15enzy µg /ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ +5µ S+1 buff
f	control	5µl 2enzy µg /ml +195µl buffer	5µl 10enzy µg /ml +195µl buffer	5µl 15enzy µg /ml +195µl buffer	5µl 20enzy µg/ml +195µl buffer	195ul buffer+5 ul buffer	5µl µg/ +19 buff

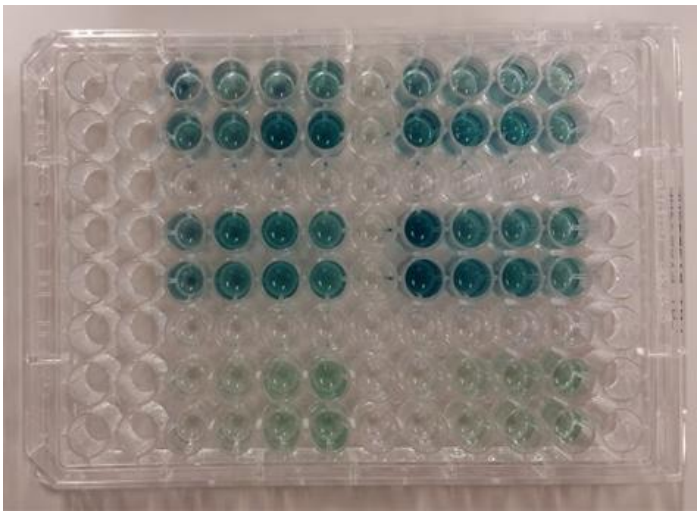
g	7	5µl 2enzy µg /ml +5µlABTS+190µl buffer	5µl 10enzy µg /ml +5µlABTS+190µl buffer	5µl 15enzy µg /ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ml +5µ S+1 buff
h	7	5µl 2enzy µg /ml +5µlABTS+190µl buffer	5µl 10enzy µg /ml +5µlABTS+190µl buffer	5µl 15enzy µg /ml +5µlABTS+190µl buffer	5µl 20enzy µg/ml +5µlABTS+190µl buffer	5µlABTS +195µl buffer	5µl µg/ml +5µ S+1 buff

Enzyme dilution prep			Step 4: Mix Enzyme dilu
pH 4		pH 5	
2	100 total volume=20microl 20 enzyme(pH4)+80microl buffer(pH4)	2	100 total volume=20mic
10	100 total volume=10microl 100 enzyme(pH4)+90microl buffer(pH4)	10	100 total volume=10mic
15	100 total volume=15microl 100 enzyme(pH4)+85microl buffer(pH4)	15	100 total volume=15mic
20	200 total volume=80microl 50 enzyme(pH4)+120microl buffer(pH4)	20	200 total volume=80mic

Step 5: In second well plate, add around 10microl of each enzyme dilution, then when assay begins, add enzyme to plate and insert into machine.

6-28_TV_assay.xlsx

6-28 plate.png

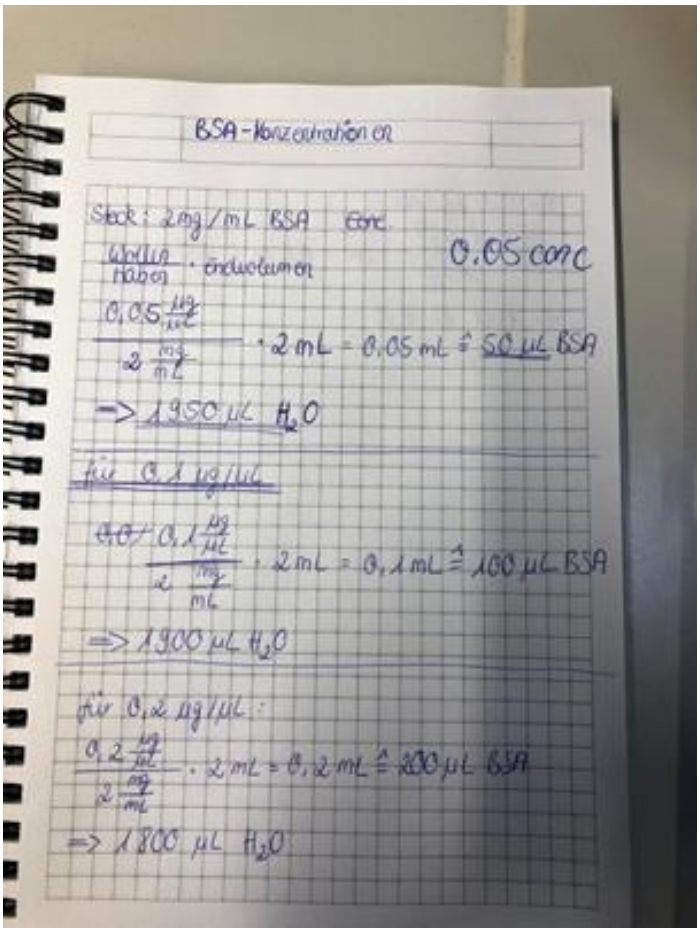


7/1/2020-Drop Assay, Lysate ABTS Assay, Bradford

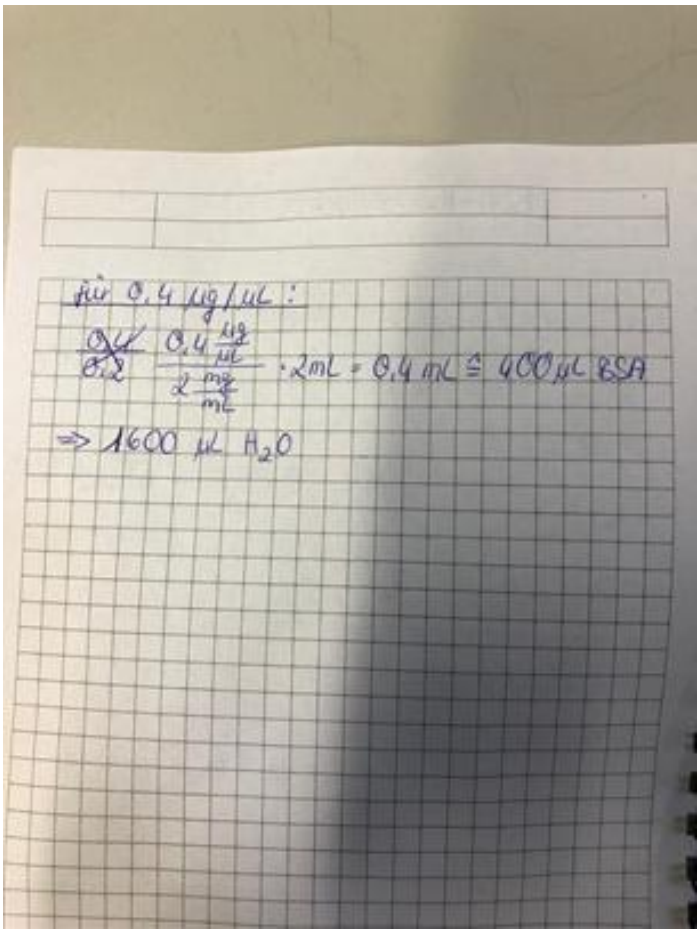
Frozen cells
pH 5 buffer
Open cells with sonification, centerfuge
Supernatent is used for lysate
More Details in "Lysate Protocoll" Document
Extract Lysate
Perform Bradford
Calculate concentration
Perform Assay
Perform Drop Assay

BSA Stock solution				
2mg/ml				
Dilutions chart				
				For Bradfordassay 20microl of each Lysate dilution needed
BSA-Stock	pH7 Buffer	Conc. (µg/µl)	µg Protein in Test	Lysate dilutions
0 µL	2000µL for 2ml	0	0	0
50 µL	1950 µL for 2ml	0.05	1	0.05
100 µL	1900 µL for 2ml	0.1	2	0.1
200 µL	1800 µL for 2ml	0.2	4	0.25
400 µL	1600 µL for 2ml	0.4	8	0.5
800µL	1200µl for 2mL	0.8	16	Undiluted

7/1 Bradford1.png



7/1 Bradford2.png

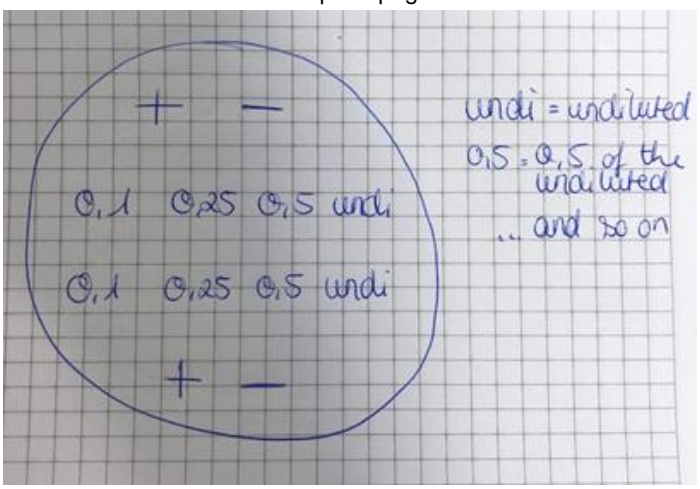


7/1/2020 Drop Test continued:

Drop Test					
Plate set up					
Positive control		UD1	0.5	0.25	0.1
Negative control		UD2	0.5	0.25	0.1
100µL of ABTS distributed on a LB plate					
Drops of 5µL Volume on each point from the corresponding dilution					

Pos control=Just Laccase at 25 dilution
Neg control=pH7 Buffer
0.1-Undiluted
10microl on each spot

Droptest.png



7/1/2020 Assay continued:

BarLac Ecoli lysate assay

Buffer		Actual after mix	
pH7		7.04	
16.47ml	3.53ml		

ABTS Dilution				
	ABTS	200µl	20µl ABTS	180µlpH7buffer

		Lysate 1				L
	Well	3	4	5	6	7
	Enzyme Concentration	0.25 UnDi	0.5 UnDi	Undiluted	0	0.
	pH					
a						
b						

c						
d	7	100 µl 0.25 UNDI µg/ml +5µlABTS+ 95µl buffer	100 µl 0.5 UNDI µg/ml +5µlABTS+9 5µl buffer	100 µl UNDI µg/ml +5µlABTS+9 5µl buffer	100 µl Buffer +5µlABTS +95µl buffer	1(5(/n +: 9:
e	7	100 µl 0.25 UNDI µg/ml +5µlABTS+ 95µl buffer	100 µl 0.5 UNDI µg/ml +5µlABTS+9 5µl buffer	100 µl UNDI µg/ml +5µlABTS+9 5µl buffer	100 µl Buffer +5µlABTS +95µl buffer	1(5(/n +: 9:
f	control	100µl 0.25 UNDIµg/ml +100µl buffer	100µl 0.5 UNDIµg/ml +100µl buffer	100µl UNDI µg/ml +100µl buffer	200 µl Buffer	1(5(/n bu
g						
h						

7-1_TV_assay.xlsx

7/8/2020-*T. versicolor* Positive Control ABTS Assay

Prepared	Basic Buffer	.2M		
	and			
	Citric Acid Buffer	.1M		
	with above buffers we made 3 different pH buffers as follows			
	pH want	.2M Na_2HPO_4	.1MCitric Acid	Actual pH
	4	15.42ml	24.58ml	3.93
	5	20.6ml	19.4ml	4.97
	7	32.94ml	7.06ml	7.03

Step 1	ABTS Dilution prep		
ABTS	200µl	20µl ABTS	180µlpH4buffer
ABTS	200µl	20µl ABTS	180µlpH5buffer
ABTS	200µl	20µl ABTS	180µlpH7buffer

OPTIONAL STEP 2	>>>Otherwise just mix ABTS and Buffer in wells separately	
BLACK mix		
Calculations for mastermix ABTS/Buffer (need one for each pH)		
32x190 Buffer (each pH)	pH4	pH5
32x5 ABTS (each buffer dilution)	160 microl ABTS buffer + 6080 microl buffer	160 microl ABTS buffer + 6

		Step 3: Fill in main wells with ABTS /Buffer for each							
		pH4				pH5			
	Well	1	2	3	4	5	6	7	8
	Enzyme Concentration	R1	R2	R3	Control	R1	R2	R3	C
	Laccase conc					Concentrations Enyzme ug per ml			

a	0	5µlABTS+195µl buffer	5µlABTS+195µl buffer	5µlABTS+195µl buffer	200µl buffer	5µlABTS+195µl buffer	5µlABTS+195µl buffer	5µlABTS+195µl buffer	
b	2	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 2enzy µg/ml +195µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	5µl 2enzy µg/ml +5µlABTS+190µl buffer	
c	10	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +195µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	5µl 10enzy µg/ml +5µlABTS+190µl buffer	
d	15	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +195µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	5µl 15enzy µg/ml +5µlABTS+190µl buffer	

e	20	5µl 20enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 20enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 20enzy µg/ml +5µlAB TS+190 µl buffer	5µl 20enzy µg/ml +195µl buffer	5µl 20enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 20enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 20enzy µg/ml +5µlA BTS+1 90µl buffer	
f	50	5µl 50enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 50enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 50enzy µg/ml +5µlAB TS+190 µl buffer	5µl 50enzy µg/ml +195µl buffer	5µl 50enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 50enzy µg/ml +5µlA BTS+1 90µl buffer	5µl 50enzy µg/ml +5µlA BTS+1 90µl buffer	
g	100	5µl 100enz y µg /ml +5µlA BTS+1 90µl buffer	5µl 100enz y µg /ml +5µlA BTS+1 90µl buffer	5µl 100enz y µg /ml +5µlAB TS+190 µl buffer	5µl 100enz y µg /ml +195µl buffer	5µl 100enz y µg /ml +5µlA BTS+1 90µl buffer	5µl 100enz y µg /ml +5µlA BTS+1 90µl buffer	5µl 100enz y µg /ml +5µlA BTS+1 90µl buffer	

h	200	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +195µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	5µl 200enzy µg/ml +5µlABTS+190µl buffer	

Enzyme dilution prep			Step 4: Mix Enzyme dilu
pH 4		pH 5	
2	100 total volume=20microl 20 enzyme(pH4)+80microl buffer(pH4)	2	100 total volume=20mic
10	100 total volume=50microl 20 enzyme(pH4)+50microl buffer(pH4)	10	100 total volume=50mic
15	100 total volume=15microl 100 enzyme(pH4)+85microl buffer(pH4)	15	100 total volume=15mic
20	200 total volume=80microl 50 enzyme(pH4)+120microl buffer(pH4)	20	200 total volume=80mic
50	200 total volume=100microl 100 enzyme(pH4)+100microl buffer (pH4)	50	200 total volume=100mi
100	200 total volume=100microl 200 enzyme(pH4)+100microl buffer (pH4)	100	200 total volume=100mi
200	200 total volume=20microl stock enzyme (pH7)+180microl buffer (pH4)	200	200 total volume=20mic

7-8_TV_assay.xlsx

7/16/2020-*E. coli* marLac and BaLac BL21 ABTS Assay

**Dilutions depend on initial volume. Need at least 300microl of each	
4 hour duration	
20microl as "undiluted" sample for T. vesticolor	

Enzyme dilution prep

2	200 total volume=2microl 200dilution+198microl buffer
200	200 total volume=20microl stock enzyme(pH7)+180microl buffer(pH7)

ABTS Dilution prep			
ABTS	300µl	30µl ABTS	270µlpH4buffer
ABTS	300µl	30µl ABTS	270µlpH7buffer

Calculations for mastermix ABTS/Buffer (need one for each pH)		
40x190 Buffer (each pH)	pH4	pH7
40x5 ABTS (each buffer dilution)	200 microl ABTS buffer + 7600 microl buffer	200 microl ABTS buffer +

	MarLac obtained:			
	65.35 microM for 750microl	Control Enzyme:T. versicolor	Purified Enzyme: MarLac	Enzy
	Well	5	6	7

	Enzyme Concentration	0.04mM	0.06535mM	0 enz
	pH			
a	7			
b	7	5µl 2enzy dilution +5µlABTS+190µl buffer	buffer	0 µl enzy 95µl
c	7	5µl 2enzy dilution +5µlABTS+190µl buffer	buffer	0 µl enzy 95µl
d	7	5µl 2enzy dilution +5µlABTS+190µl buffer	195 µl 1enzyme+5µlABTS+0 µl buffer	0 µl enzy 95µl
e	7	5µl 2enzy dilution +5µlABTS+190µl buffer	195 µl 1enzyme+5µlABTS+0 µl buffer	0 µl enzy 95µl
f	Control 7	5µl 2enzy dilution +5µlABTS+190µl buffer	195 µl 1enzyme+5µlABTS+0 µl buffer	0 µl enzy 95µl
g		5µl 2enzy dilution +0µlABTS+195µl buffer	buffer	0 µl enzy 95µl

h		Buffer	195 µl 1enzyme+0µlABTS+5 µl buffer	Buffe
			**Buffer will be substituted with incubation buffer (pH7) in this assay	

		Had airbubbles, had to remove some before putting into machine.			p o b
BaLac obtained:		Control Enzyme: T. versicolor	Purified Enzyme: BaLac	Enzyme control	
30microM for 500microl	Well	5	6	7	8
	Enzyme Concentration	0.03mM	0.03mM	0 enzyme	P c
	pH				

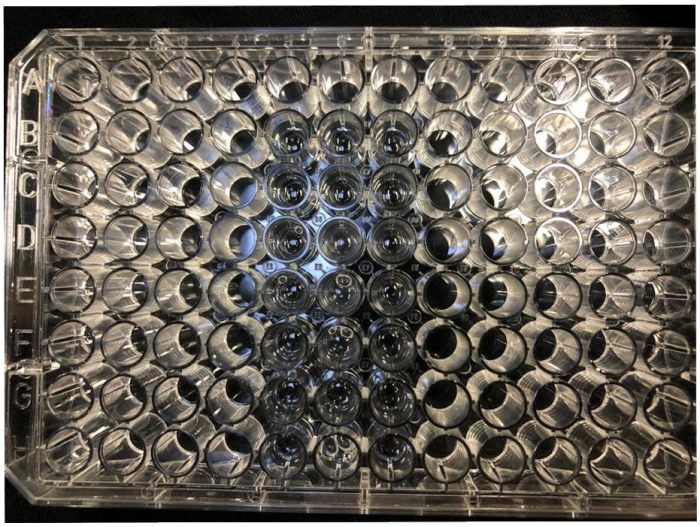
a	4				
b	4				
c	4				
d	4				
e	4	5µl Benzy dilution +5µlABTS +190µl buffer	195 µl 1enzyme+ 5µlABTS+ 0µl buffer	0 µl enzyme+5µlA BTS+195µl buffer	
f	Control 4	5µl Benzy dilution +0µlABTS +195µl buffer	195 µl 1enzyme+ 0µlABTS+ 5µl buffer	0 µl enzyme+0µlA BTS+200µl buffer	
g					

h			**Buffer will be substituted with incubation buffer (pH4) in this assay		

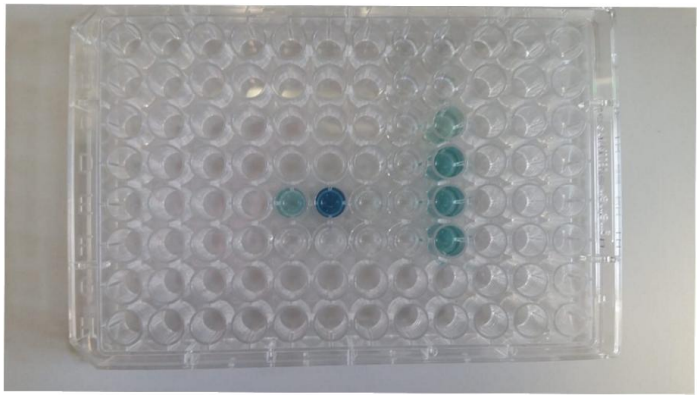
7-16_MarLac_assay.xlsx

7-16_BaLac_assay.xlsx

7/16 marLac plate.png



7/16 BaLac plate.png



7/28/2020-*E. coli* marLac ABTS Assay

Salt in process	
Salt added to increase solubility of MarLac	1M stock=584.4mg in 10mL dialysis buffer
NaCl 99.5% p.a., ACS, ISO	Adjusted to 1M stock= 578.4mg in 9.897ml dialysis buffer
M 58.44 g/mol	200microl per well, 100mM salt buffer>>20microl salt buffer in each well
Dichte 2,17	Optimal mM of NaCl according to MarLac paper=100mM
Sdp 1461C	
Carl Roth GmbH+ co	

2microg level chosen for T. ver as concentration should be around 0.04mM for made enzyme, doesn't need to be exact, just acting

4 hour duration		Tversicolor
	2	200 total volume=4microl 200dilution+196microl buffer
	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

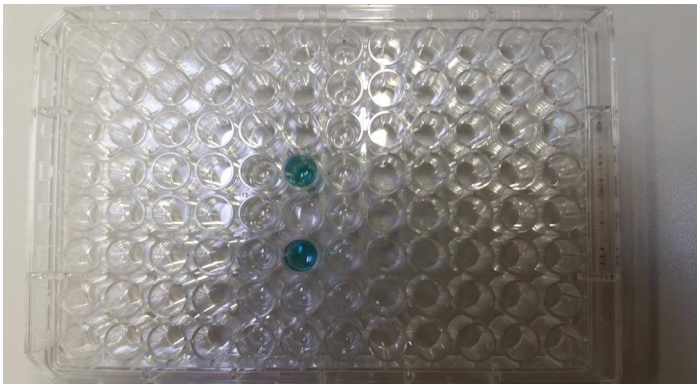
ABTS Dilution prep			
ABTS	300µl	30µl ABTS	270µlpH7buffer

	MarLac obtained:		

	28.45 microM for ~800microl	Control Enzyme:T. versicolor	
	Well	5	6
	Enzyme Concentration	0.04mM	3.57mM
	pH		
a	7+Salt		
b	7+Salt		
c	7+Salt		
d	7+Salt	5µl 2enzy dilution +5µlABTS+170µl buffer+20µl salt	5µl 200enzy dilution +5µlABTS+170µl bu
e	7+Salt-ABTS	5µl 2enzy dilution +0µlABTS+175µl buffer+20µl salt	5µl 200enzy dilution +0µlABTS+175µl bu

f	7+Buffer	5µl 2enzy dilution +5µlABTS+190µl buffer	5µl 200enzy dilution +5µlABTS+190µl bu
g	7+Buffer-ABTS	5µl 2enzy dilution +0µlABTS+195µl buffer	5µl 200enzy dilution +0µlABTS+195µl bu
h	7-Enzy	0µl enzyme +5µlABTS+175µl buffer+20µl salt	0 µl enzyme+5µlAB buffer

7/28 marLac.png



7-28_marLac_assay.xlsx

7/29/2020-Diclofenac Preparation, PreHPLC Spectra

	Tversicolor dilution
	Needs to be 200microg/ml, combining with buffer/diclo dilution at 1:1 so needs 400microg/ml
400	200microl total volume=40microl stock enzyme(pH7)+160microl buffer (pH4/5/6)

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	>>going with 20microg
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
pH4	100microl pH4 buffer+ 100microl pH4 diclo	100microl pH4 laccase+ 100microl pH4 diclo
pH5	100microl pH5 buffer+ 100microl pH5 diclo	100microl pH5 laccase+ 100microl pH5 diclo
pH7	100microl pH7 buffer+ 100microl pH7 diclo	100microl pH7 laccase+ 100microl pH7 diclo
	>>Take 100microl of each and measure in spectro after 10 min incubation	
	EACH FINAL WELL WILL HAVE 20MICROG/ML (~0.0625mM?) DICLO AND 200MICROG/ML (3.57mM) LACCASE CONCENTRATIONS	
	***We should calculate diclo's concentration from same we did for ABTS	
Dicl test with HPLC prep to verify it won't be affected by the 0.2microm filter		
Heat to 95C for 10 mins to complete reaction>>centerfuge for 5 mins at 13000		
Filter with needle, 0.2microm pore PVDF? into new epi		
Measure spectra after blanking with buffer ph5		

pH_5_Day1.xlsx

pH_5_Day2.xlsx

pH4&pH7.xlsx

pH5_HPLC_Heat.xlsx

pH5_HPLC_Heat_customized.xlsx

8/4/2020-*E. coli* BaLac BL21 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:			
	6.37 microM for 500microl	Control Enzyme: T. versicolor	Purified Enzyme:BaLac	Enzyme control
	Well	5	6	7

	Enzyme Concentration	0.04mM	0.006mM	0 enzyme
	pH			
a	4			
b	4			
c	4			
d	4			
e	4	5µl TVenzy dilution +5µlABTS+190µl buffer	195 µl 1enzyme+5µlABTS+0µl buffer	0 µl enzyme+5µlABTS+195µl buffer
f	Control 4	5µl TVenzy dilution +0µlABTS+195µl buffer	195 µl 1enzyme+0µlABTS+5µl buffer	0 µl enzyme+0µlABTS+200µl buffer
g	4			
h	4			
			**Buffer will be substituted with dialysis buffer (pH4) in this assay	

8-4_balac_assay.xlsx

8/4 BarLac plate.png