PP1 Assay

MONDAY, 8/16/2021

Well1

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Plate Blank	Substrate Blank	(-) Control	0.125	0.25	0.5	1				
C		Plate Blank	Substrate Blank	(-) Control	0.125	0.25	0.5	1				
D												
E												
F												
G												
H												

Well2

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Plate Blank	Lysate	Lysate	Lysate	Lysate	SB Lysate	SB Lysate	SB Lysate	SB Lysate		
C		Plate Blank	Lysate	Lysate	Lysate	Lysate	SB Lysate	SB Lysate	SB Lysate	SB Lysate		
D		SB No sample	WC	WC	WC	WC	SB WC	SB WC	SB WC	SB WC		
E		SB No asmple	WC	WC	WC	WC	SB WC	SB WC	SB WC	SB WC		
F		(-) control	WC_R	WC_R	WC_R	WC_R	SB WC_R	SB WC_R	SB WC_R	SB WC_R		
G		(-) control	WC_R	WC_R	WC_R	WC_R	SB WC_R	SB WC_R	SB WC_R	SB WC_R		
H												

Rxn buffer: 1720 µL = 1.72 mL

Reaction Buffer

	A	B	C	D	E	F
1	Reaction Buffer					
2		Concentration (mol/L)	MW (g/mol)	Volume (L)	Mass (g)	Mass (mg)
3	Tris-HCl	0.0625	157.56	0.2636	2.595801	2595.801
4	MnCl2	0.0002	125.84	0.2636	0.0066342848	6.6342848
5	MgCl2	0.026	95.211	0.2636	0.6525381096	652.5381096
6						
7		C1 (mol/L)	C2 (mol/L)	V2 (L)	V1 (L)	V1 (uL)
8	DTT	0.002	0.1	0.002636	0.00005272	52.72
9						
10		C1 (mg/mL)	C2 (mg/mL)	V2 (L)	V1 (L)	V1 (uL)
11	BSA	0.5	10	0.002636	0.0001318	131.8

172 uL of (TrisHCl, MnCl2, MgCl2 + 17.2 mL of H2O) + 1427 uL H2O + 34.4 uL DTT + 86 uL BSA

Stock Buffer: 1 mL total for 25 ug of PP1 (concentration of 250 U/mL)

PP1 Stock Buffer

	A	B	C	D	E	F
1	PP1 Stock Buffer					
2		Concentration (mol/L)	MW (g/mol)	Volume (L)	Mass (g)	Mass (mg)
3	NaCl	0.2	58.44	0.1	1.1688	1168.8
4	HEPES	0.05	238.30	0.1	1.1915	1191.5
5	MnCl2	0.001	125.84	0.1	0.012584	12.584
6	EDTA	0.0001	380.17	0.1	0.0038017	3.8017
7						
8		Concentration		Volume (L)		Volume (uL)
9	TWEEN-20	0.025%		0.1		25
10						
11		C1 (mol/L)	C2 (mol/L)	V2 (L)	V1 (L)	V1 (uL)
12	DTT	0.0025	0.1	0.001	0.000025	25
13						
14		Concentration				Volume (uL)
15	Glycerol	50% W/V				400

100 uL of (NaCl, HEPES, MnCl2, EDTA, TWEEN-20 + 10.0 mL of H2O) + 475 uL of H2O + 25 uL DTT + 400 uL glycerol

Enzyme Buffer

	A	B	C	D	E	F
1	Enzyme Buffer					
2		Concentration (mol/L)	MW (g/mol)	Volume (L)	Mass (g)	Mass (mg)
3	Tris-HCl	0.052	157.56	0.2	1.638624	1638.624
4	MnCl2	0.002	125.84	0.2	0.050336	50.336
5	EDTA	0.00012	380.17	0.2	0.00912408	9.12408
6						
7		C1 (mol/L)	C2 (mol/L)	V2 (L)	V1 (L)	V1 (uL)
8	DTT	0.001	0.1	0.0004	0.000004	4
9						
10		C1 (mg/mL)	C2 (mg/mL)	V2 (L)	V1 (L)	V1 (uL)
11	BSA	0.5	10	0.0004	0.00002	20

In total: 400 uL needed

4 uL DTT + 20 uL BSA + 40 uL of (20ml of (water + Tris HCl + MnCl2 + EDTA)) + 336 uL H2O

image.png

Dilution	MC-LR Stock (µL)	2.5% MeOH (µL)	[MC-LR Stock] (ng/mL)
1	11.11 (from 1E9 ng/mL stock)	100	100000000
2	11.11 (from dilution 1)	100	10000000
3	11.11 (from dilution 2)	100	1000000
4	11.11 (from dilution 3)	100	100000
5	11.11 (from dilution 4)	100	10000

MC-LR

- dissolved in 1 mL of 2.5% methanol + stored at 1,000,000 ug/mL (-20 Celsius)

PP1

- stored in stock buffer made from 200 mM NaCl, 50 mM HEPES, 2.5 mM DTT, 1 mM MnCl2, 0.1 mM EGTA, 50% glycerol, 0.025% TWEEN-20, pH 7)
- final concentration: 250 U/mL
- Stored at -80 degrees Celsius

pNPP

- Store according to manufacturer
- Prepared with reaction buffer

Enzyme Buffer

- Dilutes PP1
- 52 mM Tris-HCl (pH 7.0), 0.12 mM EGTA, 1 mM DTT (DTT in 0.01 M sodium acetate, pH 5.2), 2 mM MnCl2, 0.5 mg/mL bovine serum albumin (BSA)

Reaction Buffer

- To be used with pNPP
- 62.5 mM M Tris-HCl (pH 8.1), 0.2 mM MnCl2, 26 mM magnesium chloride, 0.5 mg/mL BSA, 2 mM DTT

How to run the assay:

Per well: 250 µL substance

- 10 µL MC-LR(0, 0.125, 0.25, 0.5, 1 µg/L)
  - Dilute in reaction buffer
  -

Stock MCLR to Working Concentration

Dilution	MCLR (µL)	2.5% MeOH (µL)	[MCLR] (µg/mL)
1	11.11 (from 1000 µg/mL stock)	100	100
2	11.11 (from dilution 1)	100	10
3	11.11 (from dilution 2)	100	1
4	11.11 (from dilution 3)	100	0.1
5	11.11 (from dilution 4)	100	0.01
6	11.11 (from dilution 5)	100	1×10^{-3}

  -

Serial Dilution MC-LR (for wells)

Dilution	MC-LR (µL)	Reaction Buffer (µL)	[MC-LR] (µg/mL)
1	40 (from 0.001 µg/mL stock)	0	1×10^{-3}
2	20 (from dilution 1)	20	5×10^{-4}
3	20 (from dilution 2)	20	2.5×10^{-4}
4	20 (from dilution 3)	20	1.25×10^{-4}

- 20 µL PP1 (0.5 U/mL, dilute in enzyme buffer)

Serial Dilution PP1

Dilution	PP1 (µL)	Enzyme Buffer (µL)	[PP1] (U/mL)
1	22.22 (from 5000 U/mL stock)	200	500
2	22.22 (from dilution 1)	200	50
3	22.22 (from dilution 2)	200	5
4	22.22 (from dilution 3)	200	0.5

- 220 µL pNPP (made with reaction buffer, 100 mM)

Plate Blanks

- 250 µL reaction buffer
- now only 200 uL

Substrate Blanks

- Just 100 mM pNPP (200 uL)

Negative control (ie. 0 µg/L MC-LR)

- Everything except the toxin

Protocols say to incubate MC-LR and PP1 together for 4 minutes and then add in the pNPP with rxn buffer.
(Altered for use of the plate reader)
(Use only 200 uL per well because of the need for extra room within eachwell)

THURSDAY, 10/14/2021

Solutions without buffer:
Reaction start: 7:21 pm

Solutions with buffer

Reaction start: 8:11 pm

Shingomonas:
Reaction start: 8:18 pm
4.95 uL of culture and 50 uL of diluted microcystin