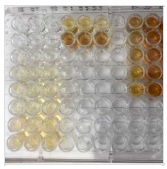

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Coral Carbohydrate Assay for 96-well plates

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ABSTRACT

1. This protocol is designed to work with coral host tissue slurry that has been processed by airbrushing to remove the tissue from the skeleton and after removal of symbiont cells and homoginization. This protocol will allow quantification of carbohydrate concentrations of samples using 96-well plates for rapid assessment.

   This protocol was adapted from Masuko et al 2005[1]. For calculation of carbohydrate concentrations, follow the directions presented in the materials section or use the custom Markdown file that can be found on my GitHub (CarbohydrateAssay_96wellplate).

1. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S, Lee YC. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Anal Biochem. 2005 Apr 1;339(1):69-72. doi: 10.1016/j.ab.2004.12.001. PMID: 15766712.

ATTACHMENTS

microcarb.pdf

DOI

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PROTOCOL CITATION

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KEYWORDS

Carbohydrate assay, Coral, 96-well plate

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MATERIALS TEXT

Reagents:

1. Concentrated Sulphuric Acid (95% certified ACS grade)
2. Phenol (certified ACS)
3. MilliQ Water
4. Glucose (L-(−)-)

Equipment:

1. 96-well plates
2. Water bath (room temperature)
3. Vortex
4. Fume hood
5. pipettes
6. plate reader (can read absorbance at 485 nm)

Coral Total Carbohydrate Calculation*:

1. Create standard curve with known standard concentrations and absorbance values ($y = mx + b$)
2. Using the resulting equation, convert sample absorbance to concentrations (mg/mL)
3. Multiply sample concentration (mg/mL) by total slurry volume (mL) and dilution factor (1000/v of sample, usually 100 mL), then divide by surface area (cm2) for resulting units: mg/cm2
4. Alternatively, you can use custom Rmarkdown script to calculate concentrations per sample well (Carb Calculation Script.Rmd) found on my GitHub (CarbohydrateAssay_96wellplate)

Carbohydrate Assay

1 Pull desired coral slurries from the -80 freezer to thaw at room temperature

2 Collect test tubes and label (*10 for the standard + the number of samples running*)

3 Make the standards and blank as shown below

Tube ID	Concentration (mg/mL)	Vol water (uL)	Vol 1 mM Glcose	Vol 10mM Glucose
B	0.0000	1000	0	0
1	0.00901	950	50	0
2	0.01802	900	100	0
3	0.02703	850	150	0
4	0.03604	800	200	0
5	0.05406	700	300	0
6	0.0901	500	500	0
7	0.1802	0	1000	0
8	0.3604	800	10	200
9	0.901	500	0	500

Standard and blank creation

4 Vortex samples after thawing (samples were previously broken up via bead beating with glass beads)

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5 Add 100 uL of coral slurry and 900 uL milliQ water to pre-labelled test tube for all samples*
You many need to concentrate or dilute your samples more depending on how they were collected.

6 Set up a room temperature water bath in the fume hood with test tube rack (DI water in a plastic bin is fine)

7 Add 25 uL of phenol to first sample

8 Vortex (in the hood) for 3 seconds

9 Immediately add 2.5 mL sulphuric acid to the sample

10 Incubate the sample at room temperature for 1 minute

11 Transfer sample to water bath

12 **Repeat steps 6-10 for all tubes**

13 When the last sample is placed in the water bath, incubate all samples for 30 minutes

14 Pipette 200 uL of all standards and samples into the bottom of the wells in a 96-well plate (see the two different plate layouts for either sets of 3 or 4 per sample)

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A	B	C	D	E	F	G	H	I	J	K	L	M
Plate layout for 3 sample replicates												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	*B*	*B*	*B*	*8*	*8*	*8*	S7	S7	S7	S15	S15	S15
B	*1*	*1*	*1*	*9*	*9*	*9*	S8	S8	S8	S16	S16	S16
C	*2*	*2*	*2*	S1	S1	S1	S9	S9	S9	S17	S17	S17
D	*3*	*3*	*3*	S2	S2	S2	S10	S10	S10	S18	S18	S18
E	*4*	*4*	*4*	S3	S3	S3	S11	S11	S11	S19	S19	S19
F	*5*	*5*	*5*	S4	S4	S4	S12	S12	S12	S20	S20	S20
G	*6*	*6*	*6*	S5	S5	S5	S13	S13	S13	S21	S21	S21
H	*7*	*7*	*7*	S6	S6	S6	S14	S14	S14	S22	S22	S22
Plate layout for 4 sample replicates												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	*B*	*B*	*B*	*8*	*8*	*8*	S4	S6	S8	S10	S12	S14
B	*1*	*1*	*1*	*9*	*9*	*9*	S4	S6	S8	S10	S12	S14
C	*2*	*2*	*2*	S1	S2	S3	S4	S6	S8	S10	S12	S14
D	*3*	*3*	*3*	S1	S2	S3	S4	S6	S8	S10	S12	S14
E	*4*	*4*	*4*	S1	S2	S3	S5	S7	S9	S11	S13	S15
F	*5*	*5*	*5*	S1	S2	S3	S5	S7	S9	S11	S13	S15
G	*6*	*6*	*6*				S5	S7	S9	S11	S13	S15
H	*7*	*7*	*7*				S5	S7	S9	S11	S13	S15

Sample plate template for either 22 total samples (9 standards, 1 blank. 3 replicate samples) or 15 total samples (9 standards, 1 blank. 4 replicate samples)

15 Cover plate and then read on spectrophotometer at 485 nm and then calculate sample concentrations following method listed under 'Materials'

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