| Protein Concentration | |
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| Prepared by: | Bahr Marine Ecology Lab |
| Last Updated: | November 2022 |
| Prerequisite SOP: Coral Airbrushing Protocol | |
| Safety Precautions: | |
| * Required PPE – Lab coat, ankle-length pants, enclosed shoes, gloves | |
| Purpose: | |
| * To determine the protein concentration of the coral host to determine coral health * Protocol adapted from Rathbone 2021, original written by Annamieke Van Den Heuvel | |
| Materials: | |
| * Spectrophotometer * SoftMax Pro Software * Computer * Thawed sample * Ice bucket * Vortex * Gloves * 15 mL centrifuge tubes * 5mL pipette & tips * 1mL pipette & tips * Kim wipes * Tip disposal * Ethanol 70% * PBS (phosphate buffer solution) * Liquid waste disposal * Quartz Cuvettes (UV transparent) * Data sheet * Pen / Pencil * Paper towels * Aluminum foil/cleaning tray | |
| Protocol: | |
| * Remove samples from freezer to thaw * Turn on spectrophotometer   + For more information on turning on the spectrophotometer see Chlorophyll A & C Extraction SOP * Open Soft Max Pro on computer * Make sure that the “SpectraMax M3” button in the top right corner on the program is a green checkmark (not red circle)   + If it’s red, click the “SpectraMax M3” button, click “COM1-SpectraMax M3”, then press “OK”   + Proceed once that button is green, red means the spec is not connected to the computer   + Drawer will open after every sample, click “Drawer” on spec to close it * Select “New Cuvette Set” * Select “Settings” on the top in the “Cuvette Tools” bar (top of the screen) * When settings open, Read Mode should be ABS, Read Type should be Endpoint   + Change Number of Wavelengths to “2” and change to Lm1 to 235 and Lm2 to 280   + Click “OK” and double check the settings saved on the right-hand corner of the screen * To start, you will need to run a reference by pressing the “Ref” on the computer (top of the screen). This solution should be 2 mL of PBS (the reference solution should be whatever suspension buffer the sample is diluted in). Use the same cuvette as the reference cuvette every time.   + You should read a reference sample after every sample for the first ~5 samples to ensure that the reference doesn’t change the previous measurement by more than ~0.005   + After the first ~5 samples, you can run a reference for every ~3-5 samples. Make sure to check that your numbers are not changing significantly, if they are run a reference after every sample * Samples will need to be diluted in the solution they are suspended in (usually phosphate buffer) to ensure that samples are above 0.1 for A280 nm and below 1 for A235 nm   + If out of these ranges’ samples will need to be diluted or concentrated as needed   + For water-piked coral samples a 1:4 dilution should work     - We were using a 1:8 dilution (0.5mL sample and 4mL PBS) - but every sample is different   + Keep track of all dilutions on data sheet   + Make the dilution in a centrifuge tube * To make the dilution, first vortex the sample to homogenize it. Then add your sample amount and PBS amount to the centrifuge tube. Last, vortex the dilution to homogenize the dilution and then pipette 2 mL into the glass cuvette. * Cover the cuvette with the white cover, invert 3 times CAREFULLY and wipe the cuvette with a kimwipe before placing into the spec   + NO liquid should be going in the spectrophotometer   + Read the samples and record all the data | |
| Analysis: | |
| Calculation:  Protein mg/mL =   * Multiply the answer by the dilution factor (i.e., 5 for a 1:4 dilution) * Finally multiply the above answer by the volume originally water-picked in (usually 50 mL) * Final answer gives total mg of protein over the surface of the coral skeleton | |
| Quality Assurance and Control: | |
| *Proper Training*  Proper protocols and training must be implemented to ensure the quality of data generated in the laboratory. Researchers must ensure that all equipment is accurately calibrated, inspected, and maintained according to the manufacturer’s instructions.  *Data Review*  All laboratory data will be reviewed for completeness and transfer errors. Data will be reviewed by a second individual after entry into Excel spreadsheets by comparing the entered, electronic data to the original records (e.g., hand-written datasheets or laboratory notebooks). Data will be summarized as descriptive statistics and in tabular and graphical form to allow visual inspection and verification, and comparison to expected or target values.    *Data Verification*  Data will be checked for compliance with the procedures outlined in the SOPs. Any deviations from those procedures and the impact on the quality of the data will be assessed and discussed with Task Members. Any laboratory data outliers will be flagged.    *Data Validation*  Once the data has been reviewed and verified, it will be assessed to determine the overall acceptability of the objectives of the project. Blank samples, such as water quality testing, will be used to determine any biases or instrument calibration issues during the sample collection and analysis processes. Control samples will be used to determine the condition of the experimental test specimens in the absence of experimental treatments or exposures. Any errors in datasets detected will be discussed with lab members and project leads to determine the impact on the data and its use for the project. If there are any limitations to the data, they will be disclosed as part of the published literature.  *Procedure Specific QA/QC Methods* Reference for protein pigments (i.e., PBS) are conducted every 5 samples to ensure the spectrophotometer is reading correctly. The spectrophotometer is inspected, calibrated, and/or maintained in accordance with the manufacturer’s instructions. All other instruments are calibrated according to calibration procedures described in the instrument manuals.  Lot numbers and expiration dates for consumables are recorded by personnel performing the testing on datasheets or logbooks, as appropriate. Reagents or standard solutions used are not used beyond the expiration date printed on the label. All supplies, equipment, and consumables procured for the analysis of this study are documented, inspected, and accepted in accordance with the requirements of each. | |