**Total Antioxidant Capacity Protocol**

Adapted from Putnam lab protocol by Taylor Lindsay

[Cell BioLabs OxiSelect Total Antioxidant Capacity (TAC) Assay Kit](https://www.cellbiolabs.com/total-antioxidant-capacity-tac-assay?gclid=EAIaIQobChMIiI6EtZK15QIVBKSzCh0uSw7pEAAYAyAAEgID-_D_BwE)

Materials

* Standard 96-Well microplate
* 1N NaOH – 1x PBS – DI water
* Methanol or other organic solvent for lipid-based samples
* Sonicator or homogenizer for samples preparations
* 10 uL to 1000 uL adjustable single channel micropipettes with disposable tips
* 50 uL to 300 uL adjustable multichannel micropipette with disposable tips
* Spectrophotometer microplate reader capable of 490 nm

Reagent Prep

1. 1x Reaction Buffer: Dilute the Reaction Buffer 1:100 with 1x PBS (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1x Reaction Buffer at 4˚C for up to 3 months.
2. 1x Copper Ion Reagent: Dilute the copper Ion Reagent 1:100 with dionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1x Copper Ion Reagent at 4˚C for up to 3 months.
3. 1x Stop solution: Dilute the stop solution 1:10 with dionized water (hydrophilic) or with methanol (lipophilic). Mix to homogeneity. Store the 1x Stop Solution at 4˚C for up to three months.

Sample Preparation

1. Samples should be stored at -80 prior to procedure
2. Thaw homogenate aliquot on ice.
3. Centrifuge at 10,000 xg for 10 mins at 4˚C
4. Aliquot the supernatant for storage at -80˚C for protein determination and subsequent TAC assay. Use this supernatant for the total protein protocol.

Uric Acid Standard Curve preparation

1. Add 10 mg of the Uric Acid powder to 1 mL of 1N NaOH (10mg/mL ratio) to create a 60 nM Uric Acid Standard.
2. Add 100 uL of the 60 mM Uric Acid standard to a 2.9 mL of DI water to create a 2 nM solution of Uric Acid.
3. Prepare a series of the remaining Uric Acid Standards according to the table below. Prepare in DI water.

Table 1.

|  |  |  |  |
| --- | --- | --- | --- |
| Tubes | 2 mM Uric Acid Antioxidant Standard uL | DI Water (uL) | Resulting Uric Acid Concentration (mM) |
| 1 | 500 | 500 | 1 |
| 2 | 500 of Tube #1 | 500 | 0.5 |
| 3 | 500 of Tube #2 | 500 | 0.25 |
| 4 | 500 of Tube #3 | 500 | 0.125 |
| 5 | 500 of Tube #4 | 500 | 0.0625 |
| 6 | 500 of Tube #5 | 500 | 0.03125 |
| 7 | 500 of Tube #6 | 500 | 0.0156 |
| 8 | 500 of Tube #7 | 500 | 0.0078 |
| 9 | 500 of Tube #8 | 500 | 0.0039 |
| 10 | 0 | 500 | 0 |

Protocol

Each uric acid standard and sample should be assayed in duplicate for replication. A freshly prepared standard curve should be used each time the assay is performed.

1. Using a pipette, mix the sample in the microcentrifuge tube. Add 20 uL of the diluted Uric Acid standards or samples to the 96-well microtiter plate.
2. Add 180uL of the 1x Reaction buffer to each well.
3. Measure an initial absorbance at 490 nm.
4. Add 50 ul of the 1x copper ion reagent into each well.
5. Incubate 5 minutes on an orbital shaker.
6. Add 50 uL of 1x stop solution to each well. This terminates the reaction.
7. Measure the absorbance for each well at 490 nm.

References

1. Ayalon, I., de Barros Marangoni, L. F., Benichou, J. I. C., Avisar, D., & Levy, O. (2019). Red sea corals under Artifical Light Polution at night (ALAN) undergo oxidative stress and photosynthetic impairment. Global Change Biology, 25(12), 4194-4207.
2. da Silva Fonseca, J., Mies, M., Paranhos, A., Taniguchi, S., Güth, A. Z., Bícego, M. C., … Bianchini, A. (2020). Isolated and combined effects of thermal stress and copper exposure on the trophic behavior and oxidative status of the reef-building coral Mussismilia harttii. Environmental Pollution , 268(Pt B), 115892.
3. Du, C., Anderson, A., Lortie, M., Parsons, R., & Bodnar, A. (2013). Oxidative damage and cellular defense mechanisms in sea urchin models of aging. Free Radical Biology & Medicine, 63, 254–263.
4. Marangoni, L. F. de B., de Barros Marangoni, L. F., Ferrier-Pagès, C., Rottier, C., Bianchini, A., & Grover, R. (2020). Unravelling the different causes of nitrate and ammonium effects on coral bleaching. Scientific Reports, Vol. 10. doi: 10.1038/s41598-020-68916-0
5. Marangoni, L. F. de B., Marques, J. A., Duarte, G. A. S., Pereira, C. M., Calderon, E. N., Castro, C. B. E., & Bianchini, A. (2017). Copper effects on biomarkers associated with photosynthesis, oxidative status and calcification in the Brazilian coral Mussismilia harttii (Scleractinia, Mussidae). Marine Environmental Research, 130, 248–257.
6. Moustafa, A. (2020). Changes in nitric oxide, carbon monoxide, hydrogen sulfide and male reproductive hormones in response to chronic restraint stress in rats. Free Radical Biology & Medicine. doi: 10.1016/j.freeradbiomed.2020.10.315
7. Strahl, J., Francis, D. S., Doyle, J., Humphrey, C., & Fabricius, K. E. (2015). Biochemical responses to ocean acidification contrast between tropical corals with high and low abundances at volcanic carbon dioxide seeps. ICES Journal of Marine Science: Journal Du Conseil, 73(3), 897–909.