**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.

*Volumes for 150 samples:*

|  |  |  |
| --- | --- | --- |
| *Reagent Name* | *Reagent Volume* | *Methanol Volume* |
| *Reaction Buffer* | *270uL* | *27mL* |
| *Copper Ion Reagent* | *75uL* | *7.5mL* |
| *Stop Solution* | *750uL* | *7.5mL* |

*Volumes for the entire kit (limited by reaction buffer 400mL):*

|  |  |  |
| --- | --- | --- |
| *Reagent Name* | *Reagent Volume* | *Methanol Volume* |
| *Reaction Buffer* | *400uL* | *40mL* |
| *Copper Ion Reagent* | *120uL* | *12mL* |
| *Stop Solution* | *1.2mL* | *12mL* |

Sample Preparation

1. Samples should be stored at -80 prior to procedure
2. Thaw host homogenate aliquot on ice.

Uric Acid Standard Curve preparation

1. Add 10 mg of the Uric Acid powder to 1 mL of 1M 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

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