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Catalase

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Eva Feldman¹, Frank Brosius²

¹University of Michigan; ²University of Arizona

Diabetic Complications Consortium
Tech. support email: rmcindoe@augusta.edu



Lili Liang

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

Describes the protocol used by the DiaComp to detect and quantify catalase activity in a tissue.

Diabetic Complications:



Cardiovascular



Nephropathy



Neuropathy



Retinopathy



Uropathy



Wound-Healing



Pediatric Endocrinology

Materials

MATERIALS

 Amplex Red Catalase Assay Kit **Molecular Probes Catalog #A-22180**

Reagent Preparation:

Amplex Red reagent: Prepare a 10mM stock solution. (Enough for 2 plates) Bring DMSO and Amplex Red reagent to room temp. Just prior to use dissolve 1 vial (.26mg) of 20mM Amplex Red reagent in 100μL of DMSO. Store stock solution at -20°C , protected from light.

Reaction Buffer (5X) (0.25M sodium phosphate, pH 7.4): Dilute 4mL of Reaction buffer in 16mL of de-ionized water.

HRP (Horseradish peroxidase) 100U/mL: Combine 15μL of 200U/mL HRP stock solution with 15μL of 1X Reaction Buffer. Store frozen at -20°C . **Reagent supplied with kit is 20U. Dissolve content with 200μL 1X Reaction buffer and divide into 22μL aliquots.**

20mM H₂O₂: (Make fresh each time.) Dilute (check bottle for %) 17.9μL H₂O₂ (3.8%) in 982.1μL dH₂O. (Check label for exact concentration) (23μL 3% H₂O₂ into 977μL dH₂O) Use promptly.

Catalase: Prepare a 1000U/mL stock. Reagent supplied with kit is 100U. Dissolve vial in 100μL dH₂O. Aliquot and store at -20°C . Make **10U/mL** with 1μL 1000U/mL stock into 99μL dH₂O. Make **1U/ml** with 10μL 10U/ml into 90μL dH₂O.

Troubleshooting



1 Sample Preparation:

Prepare Stock solution of Catalase then prepare standard curve as follows:

| Volume of Catalase stock | Volume of 1X Buffer | Final Catalase Concentration |
|-------------------------------|---------------------|------------------------------|
| 0 μL | 75 μL | 0 mU/mL |
| 18.75 μL of 1 U/mL | 56.25 μL | 62.5 mU/mL |
| 37.50 μL of 1 U/mL | 37.5 μL | 125 mU/mL |
| 7.5 μL of 10 U/mL | 67.5 μL | 250 mU/mL |
| 15 μL of 10 U/mL | 60 μL | 500 mU/mL |
| 30 μL of 10 U/mL | 45 μL | 1000 mU/mL |

(Final concentration will be fourfold lower, 0 to 10 μM)

TISSUE:

- 2 1. Homogenate tissue in 1X Reaction Buffer **on ice**.
2. Using a black plate, pipette 25 μL of diluted standards, controls (if any) and samples into wells. *(Final concentration will be fourfold lower, 0 to 10 μM)*
3. Prepare stock solution of 20mM H_2O_2 then prepare a 40 μM H_2O_2 dilution by adding 10 μL of 20mM H_2O_2 to 4.99mL 1X Reaction Buffer.
4. Pipet 25 μL of 40 μM H_2O_2 solution into each well.
5. Incubate for 30 minutes at room temp.
6. Prepare stock solution of 10mM Amplex Red reagent and divide into 50 μL aliquots and freeze immediately.
7. Prepare stock solution of 100U/ml HRP and divide into 20mL aliquots.
8. Prepare 100 μM Amplex Red reagent containing 0.4U/mL HRP by adding 50 μL of 10mM Amplex Red stock solution and 20 μL of 100U/ml HRP stock solution to 4.93mL 1X Reaction Buffer.
9. Begin 2nd phase of reaction by adding 50 μL of the above to each well.



10. Place plate into Fluroskan holder and click **"START"** .
11. Take 4 readings @ 15 minute intervals using 544/590 filter pairs. (Generally take 3 reading which would be after 30 min. incubation as recommended.)
12. Save raw data as an Excel file into the CTx data folder. Use the naming convention CTXXXX.xls, where XXXX is the date in mmdd format.
13. Select Process>Organize. Choose the appropriate data to organize (usually Measure1), then click **"OK"**. This re-arranges the data into columns.
14. Save organized data as an Excel file into the Catalase data folder. Use the naming convention ctXXXXor.xls, where XXXX is the date in mmdd format.