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Catalase

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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 an –20ºC, 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 –20ºC. **Reagent supplied with kit is 20U. Dissolve content with 200µL 1X Reaction buffer and divide into 22µL aliquots.**

20mM H_2O_2: (Make fresh each time.) Dilute (check bottle for %) 17.9µL H_2O_2 (3.8%)in 982.1µL dH_2O. (Check label for exact concentration) (23µL 3% H_2O_2 into 977µL dH_2O) Use promptly.

Catalase: Prepare a 1000U/mL stock. Reagent supplied with kit is 100U. Dissolve vial in 100µL dH_2O. Aliquot and store at –20ºC. Make **10U/mL** with 1µL 1000U/mL stock into 99µL dH_2O. Make **1U/ml** with 10µL 10U/ml into 90µL dH_2O.

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 25µL of diluted standards, controls (if any) and samples into wells. *(Final concentration will be fourfold lower, 0 to 10µM)*

3. Prepare stock solution of 20mM H_2O_2 then prepare a 40µM H_2O_2 dilution by adding 10µl of 20mM H_2O_2 to 4.99mL 1X Reaction Buffer.

4. Pipet 25µL of 40µM 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 50µL aliquots and freeze immediately.

7. Prepare stock solution of 100U/ml HRP and divide into 20mL aliquots.

8. Prepare 100µM Amplex Red reagent containing 0.4U/mL HRP by adding 50µL of 10mM Amplex Red stock solution and 20µL of 100U/ml HRP stock solution to 4.93mL 1X Reaction Buffer.

9. Begin 2nd phase of reaction by adding 50µL 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.