



CATALASE ASSAY

Version: 1

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Summary: Describes the protocol used by the AMDCC to detect and quantify catalase activity in a tissue.

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Amplex Red Catalase Assay Kit	1	Molecular Probes	A-22180

Protocol:

[Prepare reagents](#) listed above before beginning the protocol.

Sample Preparation:

1. Prepare stock solution of Catalase then prepare standard curve as follows:

Volume of Catalase stock	Volume of 1X Buffer	Final Catalase Concentration
0 μ L	75 μ L	0 mU/mL
18.75 μ L of 1 U/mL	56.25 μ L	62.5 mU/mL
37.50 μ L of 1 U/mL	37.5 μ L	125 mU/mL

7.5 μ L of 10 U/mL	67.5 μ L	250 mU/mL
15 μ L of 10 U/mL	60 μ L	500 mU/mL
30 μ L of 10 U/mL	45 μ L	1000 mU/mL

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

TISSUE:

1. Homogenate tissue in 1X Reaction Buffer **on ice**.
2. Using a black plate, pipette 25 μ L of diluted standards, controls (if any) and samples into wells. *(Final concentration will be fourfold lower, 0 to 10 μ M)*
3. Prepare stock solution of 20mM H₂O₂ then prepare a 40 μ M H₂O₂ dilution by adding 10 μ L of 20mM H₂O₂ to 4.99mL 1X Reaction Buffer.
4. Pipet 25 μ L of 40 μ M H₂O₂ 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 μ L aliquots and freeze immediately.
7. Prepare stock solution of 100U/ml HRP and divide into 20mL aliquots.
8. Prepare 100 μ M Amplex Red reagent containing 0.4U/mL HRP by adding 50 μ L of 10mM Amplex Red stock solution and 20 μ L of 100U/ml HRP stock solution to 4.93mL 1X Reaction Buffer.
9. Begin 2nd phase of reaction by adding 50 μ L 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.

Reagent Preparation: (This area may have several different preparations with the table of contents below.)

[Amplex Red Reagent](#)
[Reaction Buffer \(5X\)](#)
[HRP \(Horseradish peroxidase\)](#)
[Catalase](#)

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

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

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