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TRAP Assay

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Protocol status: Working
We use this protocol and it's working

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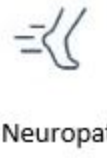
Abstract

Summary:

The use of total radical-trapping antioxidant parameter (TRAP) has recently been proposed to explore the antioxidant property of a plasma sample. This assay is a measure of oxidative stress in the animals. This protocol describes the procedure used by the DiaComp to measure TRAP.

Diabetic Complications:



Pediatric Endocrinology

Uropathy

Wound-Healing

Materials

MATERIALS

- 100 mM ABAP **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**44,091-4
- 30 mM PB pH 7.0
- Luminol **Amersham plc Catalog #**RPN2106

Reagent Preparation:

100mM ABAP*: Add 54.24 mg to 2 mL 30 mM PB.

Luminol: Mix 2 reagent ½ & ½..

Before start

IMPORTANT: **Be sure light shield is in place**

Sample Preparation - DRG:

1 1. Remove 2 DRG from vial, cut in half and weigh. Do in duplicate.

2. Add 30 µL 30mM PB and sonicate on 4 on ice.

3. Spin at maximum g's for 10 min at 4ºC.

4. Remove sup and store on ice.

Performing the Assay:

2 1. Using a **White Solid Bottom** plate, prepare plate by loading buffer for serial dilution. No PB in 1st well, 5 µL PB in following 4 wells and 5 µL in 3 control well. (Control is PB, Luminol and ABAP) (Dilutions, 1:1, 1:2, 1:4, 1:8, 1:16)

2. Prepare ABAP just prior to running assay by dissolving 54.2 mg ABAP in 2 mL PB. (Enough for 3 columns)

3. Load 5 µL sample in wells 1 & 2. Mix the sample & PB in well 2 and remove 5 µL and place in 3rd well with PB and so on. On last dilution discard 5 µL.

4. Prepare Luminol in a 50 mL conical tube and add 200 µL per well.

5. Place plate in Fluoroskan and add 60 µL ABAP per well and press **START**.

6. Read every 30 seconds for 20 minutes.

7. When reading is done, Select Process>Organize. Choose the appropriate data to organize (usually Measure1), then click **OK**. This rearranges the data into columns.

8. Save organized data as an Excel file into the TRAP Assay data folder. Use the naming convention trXXXXXX.xls, where XXXXXX is the date in yymmdd format.

*ABAP = 2,2′-azobis(amidinopropane) dihydrochloride
*TRAP = **T**otal **R**adical-trapping **A**ntioxidant **P**arameter