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Thiobarbituric acid reactive substances (TBARS) Assay

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1 Works for me dx.doi.org/10.17504/protocols.io.3sngnde

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ABSTRACT

Summary:

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are an index of lipid peroxidation and oxidative stress. The protocol describes how the DiaComp quantitates TBARS in the animal models.

Diabetic Complication:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=33&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Thiobarbituric Acid (TBA)	ICN 190284	
Trichloroacetic Acid	490-10	Sigma Aldrich
1133-tetramethoxypropane	148611000	Acros Organics

MATERIALS TEXT

Reagent Preparation:

Thiobarbituric Acid (TBA): 67 mg in 1mL DMSO then add 9 mL H_2O.

10% Trichloroacetic Acid (w/v): in H_2O.

1,1,3,3-tetramethoxypropane: 4.167 µL in 1mL Ethanol then add 49 mL H_2O. (500 µM)

Note:

Sigma-Aldrich RRID:SCR_008988

Sample Preparation:

1 **Plasma:**

• Place 100µL plasma into a labeled 1.5mL micro-centrifuge tube.

Tissue:

• Label 1 sets of 1.5mL micro-centrifuge tubes, 1 set screw top tubes and 1 set of 0.5mL tubes.

• Weighed out ~20mg and sonicate in 200µL RIPA buffer + inhibitors.

• Sonicate.

• Centrifuged @ 3000 for 10 min @ 4º .

1. Remove 10 µL aliquot into the 0.5mL tubes for protein analysis.

2. Place 100 µL lysate into a labeled 1.5mL micro-centrifuge tube.

3. Add 200µL ice cold 10% Trichloroacetic acid to precipitate protein.

4. Incubate for 15 minutes on ice.

5. Prepare standards as follows:

CONCENTRATION (µM)	H_2O	TETRAMETHOXYPROPANE
0	500	----------
0.625	500	500 from tube 3
1.25	500	500 from tube 4
2.5	500	500 from tube 5
5.	500	500 from tube 6
10	800	200 from tube 7
50	500	500 from tube 8
100	800	200 of 500uM stock

6. Centrifuge samples @ 2200 x g for 15 min. at @ 4ºC.

7. Place 200µL supernatant and standards into new labeled screw top 1.5ml tube.

8. Add and equal volume of 0.67% (w/v) TBA.

9. Incubate in a boiling water bath for 10 min.

10. Cool. Sample is ready for assay.

Performing Assay:

2 1. While samples are cooling, layout on computer and save as TBxxxxxx.sed where xxxxxx is the date in yyddmm format.

2. Load 150 µL into each standard well in duplicate.

3. Load 150 µL into each samples well in duplicate.

4. Put in plate reader and press start.

Reading the Plate:

3 • *Record absorbance at 532 nm.*

1. Turn on Multiskan and open your saved file TBxxxxxx.sed.

2. Place plate onto Multiskan holder and click **START**.

3. Select Process>Curve Fit. Choose the appropriate data (usually Measure1), then click **OK**.

4. Save Curve Fit data sheet as an Excel file into the Data folder/TBARS data folder. Use the naming convention TBxxxxxx.xls, where xxxxxx is the date in yymmdd format.