Thiobarbituric acid reactive substances (TBARS) Assay

Version: 1
Replaced by version: N/A
Edited by: Eva Feldman, M.D.

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

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Thiobarbituric Acid (TBA)	ICN		190284
Trichloroacetic Acid	Sigma		490-10
Malonaldehyde bis(dimethyl acetal)	Fisher		AC 14861-1000

Protocol:

Reagent Preparation:

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

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

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

Sample Preparation:

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 @ 4^0.

1. Remove 10µL aliquot into the 0.5mL tubes for protein analysis.
2. Place 100µL 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 @ 4^0C.
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:

1. While samples are cooling, layout on computer and save as TBxxxxxx.sed where xxxxxx is the date in yyddmm format.
2. Load 150µL into each standard well in duplicate.
3. Load 150µL into each samples well in duplicate.

4. Put in plate reader and press start.

Reading the Plate:

- Record absorbance at 532nm.

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.