Texas Tech University

Thiobarbituric Acid Analysis (TBA)

1. Immediately following end of display period, vacuum package the steak and keep it sealed until use.

2. Make all reagents and **refrigerate** for future use.

   TCA (Trichloroacetic acid) –(20%) H_3PO_4 (2M)
   Bring 200 g TCA and 136 mL Phosphonic acid to 1 L volume with distilled water.
   ***Weigh TCA in glass beaker, not plastic weigh boats, as TCA dissolves plastic*

   TBA (Thiobarbituric acid) (.005m)
   Add .7212 g TBA to 1 L distilled water. Heat on hot plate to bring into solution before use.

   TEP (Tetraethoxypropane) (4.046 x 10^{-5} M)
   Bring 10uL TEP to 1 L with distilled water.
   **(10uL/L)(.000919 g/uL)(.97% pure)/(220.3 g/mole)=4.046 x 10^{-5} M*
   **TEP is used as a standard for malondialdehyde (MDA) equivalents (1 mole TEP=1 mole MDA in reacting with TBA)*

3. Weigh approximately 10 g of sample. Homogenize sample and 50 mL distilled water for 1 min.

4. Add 50 mL chilled TCA-H3PO4 solution and homogenize again for 15 sec.

5. Transfer 30 mL homogenate to a 50 mL centrifuge tube. Keep sample refrigerated before homogenizing and keep homogenate sample on ice.

6. Centrifuge at 10,000 x g for 20 min. in refrigerated centrifuge. (9000 x g on Beckman centrifuge).

7. Filter through #1 filter paper.

8. Pipette 4 mL of filtrate and 4 mL TBA solution into 13 X 100mm test tubes in duplicate. Cap tubes with rubber cork and vortex.

9. Pipette set of standards to include in each batch of samples. One replication per standard. Cap and vortex.

10. Standards

Standard #	MDA	mL TEP solution	mL TBA solution	mL TCA solution	mL water
1	.0000	0	4	2	2
2	.1011	.02	4	2	1.98
3	.2023	.04	4	2	1.96
4	.4046	.08	4	2	1.92
5	.6069	.12	4	2	1.88
6	.8092	.16	4	2	1.84
7	1.0115	.20	4	2	1.8
8	1.2138	.24	4	2	1.76
9	1.6184	.32	4	2	1.68
10	2.023	.4	4	2	1.6
11	2.529	.5	4	2	1.5
12	3.0345	.6	4	2	1.4
13	3.540	.7	4	2	1.3
14	4.046	.8	4	2	1.2
15	4.5512	.9	4	2	1.1
16	6.069	1.2	4	2	.8

***A pre-set of standards can half way be made ahead of time and kept dark and cool. Multiply the mL TEP X 20 and the mL water X 20 and mix in a conical for each of the 16 standards. Then, on the day your batches are being prepared, add the appropriate amount of TBA and TCA to the 16 13 x 100mm test tubes along with 2 mL of the pre-made standards from the corresponding conical 1-16.

11. Keep samples and standards in dark at room temperature for 15 h.

12. Vortex and read absorbance at 533 nm (visible). Read 16 standards first and then the rest of the batch since the spec program is set-up to analyze in this order. **Try to be consistent in the time samples are kept in the dark before measuring Optical Density (OD) for samples analyzed on different days*.

13. Use the spec program - under PROTEIN there is a program written for TBA.