Protocol 6: Fe-BABE Modification of RNA with 5'-Phosphorothioate.

1. Mix 20 μl of 28 mM freshly made ammonium iron (II) sulfate hexahydrate (Aldrich#20350-5) with 25 μl of 28 mM BABE.

2. Adjust pH to 3-4 using 1-2 μl diisopropylethylamine (pH 9). Use pH paper to verify pH.

3. Incubate at room temperature for 1 hour.

4. Combine:

5'-GMPS-RNA	= μl	(800 picomoles)
12 mM Fe(II)-BABE mix (from step 3)	= 5.0 μl	
400 mM Potassium phosphate (pH 8.5)	= 1.0 μl	
Water	= μl	

	10.0 μl Final volume	
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5. Incubate at 37 °C for 1 hour.

6. Add 1.0 μl of 50 mM EDTA. Incubate for 10 minutes at 37 °C.

7. Add 10 μl of 5M ammonium acetate, 1 μl of 10mg/ml glycogen and 80 μl of water.

8. Extract with 100 μl of water-saturated Phenol. Transfer supernatent to new tube.

9. Repeat Phenol and then chloroform extractions. Transfer supernatent to new tube.

10. Add 300 μl of 100% ethanol. Place in –80 °C for 1 hour or –20 °C overnight.

11. Spin tubes in microfuge at max speed for 30 minutes at 4 °C.

12. Carefully remove ethanol without disturbing the pellet. Dry the pellet in SpeedVac.

13. Resuspend the RNA pellet in water at 25 picomoles/ μl concentration.

14. Store Fe-BABE-RNA at –20 °C and use it within 2-3 weeks for tethered hydroxyl radical probing experiments.

Note: We currently have following stocks of BABE:
(a) 28 mM BABE in 20 μl aliquotes synthesized on 8/7/95
(b) 49.6 mM BABE in 20 μl aliquotes synthesized on 4/22/97.