

## PREPARATION OF AET/SRBC

AET = 2-Amino-Ethyl Isothirouronium Uronium Bromide Hydrobromide

SRBC = sheep red blood cells

1. Wash with Normal Saline, 15-20 ml sheep red blood cells in a 50. ml centrifuge tube 3 times at 1200 rpm for 10 minutes. Wash and spin a 4th time for 10 min. at 1800 rpm after transferring SRBC to a 15 ml centrifuge tube.
2. Weigh 0.40 grams AET and add to 10.0 ml deionized H<sub>2</sub>O. Adjust ph to 8.9. 0.10-0.1ml 2N NaOH should be close to 8.9
3. Remove buffy coat from packed SRBC after 4th spin.
4. Add 2.0 ml packed SRBC to 8.0 ml AET solution in a 50.0 ml tube.
5. Mix gently and incubate in 37°C H<sub>2</sub>O bath for 15 minutes.
6. Wash 4 times with cold saline and once with M&D balanced salt solution (BSS) with 10% FCS.
7. Resuspend in 10% BSS and count SRBC. Store in refrigerator for up to one week.

### NOTES:

1. Prepare AET/SRBC in a sterile manner.
2. If you need to prepare a larger amount of AET/SRBC, increase numbers of tubes in step 1 and weigh out more AET and add appropriate volume of deionized H<sub>2</sub>O.