T CELL ROSETTING WITH AET/SRBC

AET = 2-amino-ethyl isothiouronium bromide hydrobromide

SRBC = sheep red blood cells

PREPARATION OF AET/SRBC

1. *Wash qs for 10mls packed SRBCs with cold, sterile normal saline in a 50ml tube 4 x 10' ea. @ 1500rpm.*

2. *For each 2mls of packed SRBCs weigh 0.40 grams AET and add to 10mls water. Adjust to pH 8.9 w/ 10N NaOH and sterile filter.*

3. *Add 2.0mls packed SRBCs to 8.0mls AET solution in a 50.0ml tube.*

4. *Mix gently and incubate @ 37 C x 15'.*

5. *Wash 4 x 10' ea. w/ cold saline and once more w/ cold RPMI or balanced salt solution + 10% FCS.*

6. *Resuspend in approx. 50mls cold R10 and count a 1:1000 dilution.*

7. *Store AET/SRBCs @ 4 C up to one week.*

T CELL ROSETTING

0. *ALL STEPS @ 4 DEGREES C.*

1. *Mix 1 x 10(8) buffy coat MNCs + 1.5 x 10(10) AET/SRBCs in a 50ml tube. Spin x 5' @ 1000rpm @ 4 C.*

2. *Incubate @ 4 C x 1.5hrs to o.n.*

3. *Dilute to 45mls w/ R10. Pipette w/ 25ml pipette to seperate rosettes from non-Ts.*

4. *Layer 15mls over 15mls ficol-hypaque. Spin 20' @ 1200rpm, increasing to 3000rpm for the final 5'.*

5. *Recover the non-Ts from the interface and wash 3 x 10' ea. w/ R10.* *

6. *Expect 10-20% recovery of number rosetted.*

* Lyse contaminating SRBC's by suspending 1° pellet in 2-5 mls Tris-NH_4Cl for 3-5 min. Suspension will change from opaque to transluent red. continue w/ ~~sp~~ step 5 washes. Pellet should be white. Repeat if needed.

a) Tris: 20.6 gm Tris base/l pH 7.2 - 7.4

b) NH_4Cl: 8.3gm/l c) Working Tris/NH_4Cl A:B = 1:9