Blood Leukocyte Isolation and RBC lysing for RNA

1. Collect blood direct from the sheep into a jug with ACD to prevent clotting (10ml of ACD per 100ml of blood).
2. Transfer to 2x50ml falcon tubes.
3. Centrifuge @ 500 x g for 10 minutes (no brake) @ room temperature.
4. Pool the buffy coats from each tube into 15ml falcon tubes (no more than 1ml of buffy coat per tube).
5. For 1ml of cells add 14ml diluted lysis buffer.
6. Rock for ~10 minutes @ room temperature until liquid is clear red.
7. Centrifuge at 4°C for 5 minutes at 500 x g.
8. Decant supernatant, pipette off any excess supernatant and allow tubes to drain briefly.
9. Add 1ml of RNAlater and resuspend the pellet then add an extra 4ml of RNAlater and mix by pipetting.
10. Freeze the lysate in 1ml aliquots in screw top 1.5ml eppendorfs in the -80°C freezer (2x50ml falcon tubes should give 5 aliquots).

Ammonium chloride lysis buffer (10x concentration)

NH_4Cl (ammonium chloride) 8.02g
$NaHCO_3$ (sodium bicoarbonate) 0.84g
EDTA (disodium) 0.37g
Make up to 100ml with Millipore water

Working solution

Dilute 10ml 10x concentrate with 90ml Millipore water

Store lysis buffer and working solution at 4°C until use.