

Lysis of Red Blood Cells

Tris-buffered ammonium chloride

Stock solutions:

1. 0.16M NH₄Cl: 8.3g/liter (**4.28g/0.5L**)
2. 0.17M Tris (Hydroxymethy-aminomethane), pH 7.65: dissolve 20.6g Tris base in 900ml water (**2.06g/100ml**)
3. Adjust to pH 7.65 with HCL
4. Make up to 100ml

Working solution:

1. Mix 90ml of 0.16M NH₄Cl and 10ml of 0.17M Tris, pH 7.65
2. Adjust to pH 7.2 with HCL (**450ml NH₄CL + 50ml Tris**)

Lysis of red blood cells:

1. Pellet the cells and resuspend in Tris-NH₄CL working solution (0.1ml packed cells/ml Tris-NH₄Cl). Hold at room temperature for 2 minutes.
 - (For one spleen, use 2ml Tris-NH₄Cl.)
2. Underlay the cells with FCS and centrifuge at 300g for 10 min.
3. Repeat the process if red blood cells are evident in the pellet.
4. Wash cells twice with RPMI 5% FCS.