Red blood cell (RBC) lysis buffer – working stock:

Dissolve the following in 1 liter ddH_2O:

8.26 g ammonium chloride (NH_4Cl)
1 g potassium bicarbonate ($KHCO_3$)
0.037 g EDTA

Mix well and autoclave

Store up to 6 months at room temperature or 4°C
RBC lysis buffer should always be used at room temperature.

Ficol w/ lysis:
We would ficol prep 15-20 ml of human blood and after washing 1X with PBS add 30-40 ml lysis buffer incubating mix 5 minutes maximum @ up to 37C. I liked using the rocker for 2-3 minutes. **The faster you get the cells out the better.**

Staining whole blood:
For staining whole blood, we added the Abs to 100-250ul of heparinized blood which incubated 30 minutes on ice. Run these samples ASAP no fix or follow up with RBC lysis below and store up to 2 weeks. (Whole blood doesn't fix or store well.)

Lysis following staining of whole blood:
To lysis RBCs in whole blood, we then added 1-4 ml lysis buffer warmed to 37C (fresh lysis quickly with less than stuff that's stored). 3 min @ 37C. Centrifuge 3-5 minutes, decant supernatant and resuspend pellet in 1X PBS + 2-5% serum. Centrifuge again and resuspend in 4% paraformaldehyde.

We did 37C lysis because cells were on ice during previous step – so this sort of brought them up to RT.