Creighton
UNIVERSITY

Tris-Buffered Ammonium Chloride (ACT) for Red Blood Cell Lysis

(Greg A. Perry, Ph.D.)

Equipment:

Glass Beaker
Stir Plate & Stir Bar
pH Meter
500ml Glass bottles (x3)

Reagents:

Stock Solution A

4.15	gm	Ammonium Chloride (NH_4Cl) (Sigma #A5666)
500	ml	Distilled Water

Stock Solution B

10.3	gm	Trizma Base (Sigma #T6066)
500	ml	Distilled Water
		2N Hydrochloric Acid (HCl)

Other Reagents

2	N	HCl

Method:

Stock A

1) Dissolve Ammonium Chloride in distilled water for a 0.16 M solution

Stock B

1) Dissolve Tris Base in 450 ml distilled water.
2) Adjust to pH = 7.65 using 2N HCl.
3) Adjust to 500 ml final volume with distilled water.

Working Solution

1) Mix 450 ml of Stock A with 50 ml of Stock B.
2) Adjust to pH = 7.2 using 2N HCl.

Note: *For some reason which is not entirely clear, we have found that using Tris Base and Ammonium Chloride that are intended for molecular biology use do not seem to work for this protocol. Please be sure you are NOT using chemicals marked as "For Molecular Biology".*

From: ***Selected Methods in Cellular Immunology****, B. Mishell & S. Shiigi, Eds, (W.H.Freeman: New York), pg. 23, 1980.*