Staining of cells with PI using Hypo/Hyper Buffer

Protocol note: this procedure does not require fix/perm with ethanol. The high concentration citrate in hypotonic conditions will cause the cells to burst open, allowing PI access to the nucleus. Hypertonic solution restores isotonicity. Evaluation is on nuclei only (take into account for FSC/SSC setup).

Protocol:

1. Add 250 µL of Hypotonic Stain Buffer
2. Add 25 µL of RNase A (final conc 800 U/ml)
3. Mix and incubate at 37°C for 45 min
   (For the Flow course we will drop to 15 minutes)
4. Add 250 µL of Hypertonic Stop buffer,
5. Incubate at 4°C for > 1 hour, but < 48 hours, analyze on cytometer
   (For the Flow Course we will incubate for 15 minutes)

Needed:

- FCM buffer
- 10% Triton X-100 in FCM buffer
- PI stock 1 mg/ml: Dissolve 10 mg PI and 0.3 g PEG in 10 ml PBS (good for 1 year at 4°C protected from light).
- Rnase A stock: 8,800 U/ml. Stock 2.5mg/ml 10/2012 = 23,325 U/ml (solid 9,330 U/mg). Take 1 ml 2.5 mg/ml stock + 1.65 ml PBS for 8,800 U/ml stock.
- 0.4 M NaCl stock: 1.169g NaCl in 50 ml DI water
- 4 mM Na_3Citrate stock: 59 mg Na_3Cit in 50 ml DI water, pH to 7.8.

Hypotonic Stain buffer:	FC	for 10 ml:
Sodium citrate	3.6 mM, pH 7.8	9 ml 4 mM stock
Propidium iodide	50 µg/mL	0.5 ml 1 mg/ml stock
PEG 4000	3%	0.3 g
Triton X-100	0.1%	100 µl 10% stock
Water		0.4 ml

Hypertonic Stop solution:		
NaCl	0.375 M	9.4 ml 0.4 M stock
Propidium iodide	50 µg/mL	0.5 ml 1 mg/ml stock
PEG 4000	3%	0.3 g
Triton X-100	0.1%	100 µl 10% stock