General Lineage Depletion Procedure (for HSC enrichment from bone marrow)

PROCEDURE:

1. Remove bones, clean and crush in ice cold HBSS 2% FBS (HBSS+) in a mortar and pestle.

2. Filter cells through 40µm filter to remove bone fragments, pellet cells at 380 x *g* 4º C for 4 minutes.

3. Resuspend in 1 mL ACK red cell lysis buffer, 5 minutes on ice then stop by adding 5 mL HBSS+, pellet cells at 380 x *g* 4º C for 4 minutes.

4. Resuspend in 5 mL HBSS+, count at 1:20 dilution in trypan blue.

5. Pellet cells at 380 x *g* 4º C for 4 minutes. Resuspend cells to be at a final concentration of $5x10^7$/ mL.

6. Stain cells using 1µL lineage mix/ 1 million cells. Note: for different cell populations and different antibodies, a titration of all antibodies should be performed to determine optimal binding of all lineages. Staining volume can be between 50 million/mL to 100 million/mL.

Lineage Mix (all from Caltag [now Invitrogen] except IL-7Rα=eBiosciences)

CD3	CD8	CD19	Ter119	CD4	Gr-1	B220	Mac-1*	IL7Rα*
1µL	1µL	1µL	1µL	2µL	2µL	2µL	2µL	2µL

*=optional depending on application

6. Wash cells 2x with 2-4 mL HBSS+, pellet cells at 380*x g* at 4º C for 4 minutes.

7. Resuspend cells in 7 mL HBSS+ per $5x10^7$ cells then add 100-150µL Dynal sheep anti-rat (102-06) beads. Volume matters here. Dynal beads should be washed and adjusted to the appropriate cell:bead ratio according to manufacturer's recommendations. Nutate (mix using a rocking platform) in a sealed tube at 4º C for 25 minutes.

8. Deplete $lineage^+$ cells by incubating FACS tube in Promega MagneSphere® Technology Magnetic Separation Stand (Z5333) for 2 minutes. Remove $lineage^{neg/low}$ cells with Pasteur or other skinny pipette tip.

9. Spin down at 380 x *g* at 4º C. Wash cells with 4 mL HBSS+ then resuspend in 1 mL HBSS+ and count cells at 1:10 dilution in trypan blue.

- Expect 10-20% of starting cell number post depletion and ~ 60-80% purity. Yield is better than other commercial kits but purity makes this technique

appropriate as a pre-sort step.

- For detection of unlabeled antibodies, we use Caltag Goat $F(ab')_2$ goat anti rat IgG PE-Cy5.5 at a concentration of 2 µL per 10^7 cells (Cat #R40118).

REAGENTS:

ACK lysis buffer
0.15 M NH_4Cl
1.0 mM $KHCO_3$
0.1 mM Na_2EDTA
pH 7.2-7.4

HBSS+
500 mL Hank's Buffered Saline Solution
10 mL FBS
antibiotics optional

antigen	company	cat. #
IL7Rα	ebioscience	14-1271-82
Mac-1	caltag	RM2800
B220	caltag	RM2600
Gr-1	caltag	RM3000
CD4	caltag	MCD0400
Ter119	caltag	mTER00
CD19	caltag	RM7700
CD8	caltag	MCD0800
CD3	caltag	RM3400