

## **BD Cytofix/Cytoperm Kit for Intracellular mouse NK Staining**

1. **Surface stain** in U-bottom 96 well plate with 1-2 x10<sup>6</sup> cells/test in 100-200 ul total volume of FACS buffer. (Alternatively, may stain in flow tubes):
  - a. Add FcBlock (0.25 ul / test or 2.4G2 hyb supe); may FcBlock prior to plating, wait 0-5 min [this blocks non-specific surface staining by binding CD16/32]
  - b. Add surface staining antibodies, mix, incubate 15 min 4°C, dark
  - c. Wash 1X w/ FACS buffer (spin 5 min at 1200 rpm, flick plate, resuspend in FACS buffer)
2. **FIX:** Re-suspend in 200 ul BD Cytofix/Cytoperm (1X, brown bottle) solution, incubate 20 min (minimum) to hours in 4°C in dark; do not leave overnight!

### **IF STOPPING AT THIS POINT:**

3. Wash 1X w/ FACS buffer
4. Resuspend in FACS buffer, 200 ul / well; may stop at this point and resume IC staining later (typically within 72h)
5. To resume IC staining, wash 1X w/ Perm/Wash and continue next step 7 (Block)

### **TO CONTINUE ON WITH IC STAINING:**

6. Wash twice with BD Cytoperm/Cytowash (10X stock, dilute to 1X in ddH<sub>2</sub>O)
7. Resuspend in blocking perm/wash (perm/wash with 7.0% goat serum added); incubate 30 min, 4°C, dark
8. Stain with antibodies to intracellular proteins (e.g. anti-perforin, granzyme, IFN- $\gamma$  mAbs, mix, incubate 30 min, 4°C, dark)
  - a. For Gzm B (0.25 ul/test) – clone GB12 (Caltag) [titer on IL-15-activated NK cells each lot]
9. WASH 2X w/ BD Perm/Wash
10. Resuspend in 200-1000 ul of FACS buffer, transfer to flow tubes
11. Acquire data

### **FACS buffer = Ca/Mg-free PBS, 1mM EDTA, 2% FCS**

Add to Ca/Mg Free PBS (“Solution A” from tissue core)

For 1L: 2 mL 0.5 M EDTA, 20 mL FCS (heat inactivated)

For 0.5L: 1 mL 0.5 M EDTA, 10 mL FCS (heat inactivated)

### **Reagents:**

BD Cytofix/Cytoperm Fixation/Permabilization Catalog No:554714

Fc Block (BD, alternatively can use 2.4G2 hybridoma supernatant or purified antibody)

Flow tubes

96 well U bottom plates for flow

IL-15 [Peprotech] (Cf: 100 ng/mL) B6 splenocytes x 48-72 hours, gating on NK1.1+CD3- cells

### **Notes:**

Use 12 multichannel pipette when doing large numbers of samples in plates to save time

Make cytoperm/cytowash dilution fresh

Anti-GzmB (clone GB12) requires titrating on each lot using IL-15 activated splenocytes. Titer is typically approximately 0.25 ul/test.

Acquire data within 24 hours for best results