Flow cytometry staining protocol for LYVE1 Antibody (NBP1-43411AF405)

1. Harvest, wash the cells (single cell suspension) and adjust cell number to a concentration of 1-5x106 cells/ml in ice cold FACS Buffer (PBS, 0.5-1% BSA or 5-10% FBS, 0.1% NaN3 sodium azide*).
   Cells are usually stained in polystyrene round-bottom 12 x 75 mm BD Falcon tubes (cat # 352052). However, they can be stained in any container for which you have an appropriate centrifuge e.g. test tubes, eppendorf tubes, and 96-well round-bottomed microtiter plates. It is always useful to check the viability of the cells which should be around 95% but not less than 90%.

2. Add 100 µl of cell suspension to each tube.
3. The blocking antibody step 3 is optional but should be included if cells express high levels of Fc receptors which will contribute to non-specific binding and background fluorescence.
   Add 100 µl of Fc block to each sample (Fc block diluted in FACS buffer at 1:50 ratio). Incubate on ice for 20 min. Centrifuge at 1500 rpm for 5 min at 4°C. Discard supernatant.
4. Add 0.1-10 µg/ml of the primary labeled antibody.
   Dilutions, if necessary, should be made in FACS buffer.
5. Incubate for at least 30 min at room temperature or 4°C in the dark.
   This step will require optimization.
6. Wash the cells 3 times by centrifugation at 1500 rpm for 5 minutes and resuspend them in 200 µl to 1ml of ice cold FACS buffer*. Keep the cells in the dark on ice or at 4°C in a fridge until your scheduled time for analysis.
   Analysis: for best results, analyze the cells on the flow cytometer as soon as possible.
   We recommend analysis on the same day. For extended storage (16 hr) as well as for greater flexibility in planning time on the cytometer, resuspend cells in 1-4% paraformaldehyde to prevent deterioration.