Staining T cell hybridomas

1. We use a staining buffer consisting of : Balanced Salt Solution + 2% Fetal Calf serum + 0.05% NaN_3 = "BSS Wash-Buffer".
2. In microtiter plate wells combine 50 ul cells (~ 1x 10^6 cells) in staining buffer + 10 ul 2.4G2 supernatant (to block Fc receptors) + 30 ul staining antibody.
3. Incubate 30 minutes, on ice, covered.
4. Add 60 ul BSS Wash buffer.
5. Spin 1x, 4 minutes, 1500 rpm, 380 g , 4^oC.
6. Discard supernatant.
7. Shake plate ~ 3 seconds.
8. Add 150 ul BSS Wash buffer.
9. Spin 1X, 4 minutes, 380 g, 4oC.
10. Discard supernatant.
11. Resuspend cells in 300 ul BSS Wash buffer and transfer into staining tubes.