FoxP3 intracellular staining on microtitter plates

eBioscience Kit

1. We put the cell suspension on microtitter plate by $1x10^6$ cells/well.
2. 1x wash with 200µl PBS (400g, 2min., 4ºC)
3. Stain surface antigens (see Surface staining)
4. 1x wash with 200µl PBS
5. shake
6. resuspend cells in 50µl fresh Fixation/Permeabilization buffer:

   Preparing Fix./Perm, buffer:

   1 unit of Fixation/Permeabilization Concentrate + 3 units of Fixation/Permeabilization Diluent. (e.g. 100µl +300µl)

7. incubate 30min. 4ºC
   1x wash with 100µl 1x permeabilization buffer

   Preparing 1x permeabilization buffer:

   1 unit of 10x Permeabilization Buffer + 9 units of dd H_2O
   (e.g. 1ml 10x Perm. Buf. +9ml ddH_2O)

8. Block cells with 50µl 2% rat serum diluted in 1x perm. buffer
9. Incubate 20min 4ºC
10. Centrifuge, aspirate liquid
11. Dilute FoxP3 antibody at given titter in 1x perm. buffer
12. Stain with diluted FoxP3 antibody by 10µl per well
13. Incubate 30min., 4ºC, in dark
14. 2x wash with 200µl 1x perm. buffer
15. Resuspend cells in 100µl 1x perm. buffer
16. analyse