Protocol 60_I naeueClykir SgtiF

In Vitro WasWh/P PemiWWtasWasWtoWmaVWtWWaWtoWroisW W

- *VitroWasWWmirWtoWWmiWsaWtaVeWWWW* wgmR
  tCms c:CmMg*WtaW*gSSiryhg*WmaretWWWtoWtVaWtesWisWtoW*
  h/P PemiW V W
- *VitroWasWraWVWtasWasWtoWraWesWtasWasWtoWn W*
- *itWWeWWWWtaWVeWWWWWesWW*
  itWWWsatWrWtaW/WesWVeitWeWWW W
- *WVaVitoW/P meWW nWestiWWVimmWoeWtaWWtoetW*
  *to*dyNg OglCT eClykrCtyom*eWtmWWWWerWW*
  n W
  PeW/WasWeWWVaWVitoW/P meWWestiWWressatWW
  ramaW meWWWestiWesWeWato WoWWesWtoWmW
  meWertiWtoWWP meWWWestiWisWWasmW
  ertiWtoWWmeW W
- dyNg OglCT eClykrCtyom 1oFtyomCl. iSiCllT omlT FgrporNg2 kT cirCtoWAE

e. *Wh/P Pa W*

- *raWtWeWW* accgFt *as W*

r. *WisWtiasWeWroWVoW* eClykrytg /gC2 Dot %*WsWtoW*
WeWtoWWeWasWtoWPemiWWWWesWmWrWWsatW
tWPemiWWtWWeWtsWasWtoWir W

- *W* (im *as W*
- *himmWh/P WWroWVitoWn/nWesWIWWaWWWh/P WaW*
  oetoWhmWesWeW W

nWIWWaWmeWWWesWIWWaW/P meWW W
WIWWaWmeWWWesWIWWaW/P meWWWesW IWWaWroWaW
hP W WsWP meWW W
oeW tWVmmWWeWtoWWesWWtoetWW
maaWrmaW W

W

- *WW/P WsWmeWPemiWWtW/nWasWtoWraWesWrmirW*
  *to*utCrt *as W*
- *h/P PaWVimmWtW/P WsWmeWWWesWroWVoWata W*
  ntimiWWWeWWrmWWtoiWiWtoWreWeWWVimmW
  eWWW P5dS ugt uiccgSSpillT *W*
- *WWh/P PaWVimmWeWtiremmWWasWVitoWWmeWPemiWtiasWVitoaW*
  oeWtaWWesWtasWWVimmWeWWW PgrporNymM dyNg
  OglCT eClykrCtyom*WWWmeWPemiWtiasWiWrWesatoWW*
  VimmWeWWW dyNg OglCT eClykrCtyom µCS SiccgSSp*W*
- *WWmeWPemiWtiasWVeWrWWWWWWW*
  WWtW WasWtW WsWrmirW/W/mmaVWtoWroisWtaWW
  toWitWWWtaramW W
  - *WtoWratWtWiWsatWWaWWWisrWtoWWtaWWaW*
    WasWtoWstWsWaWtoWraW W
  - *WWtoWWWsiWWVimmWWeW WamaWW eWh/P WW*
    WresWtWtoiWWisWtaWhimWesWmmisWWVsWtaWtW
  - *WWaWtWWtWWtaWWesWWrWtoWWtW W*
  - *PmirWW W*

o. *VWrmir*gll)igSt ProB *Pmir*ylg*WWsW* 2ociNgmtB*WtaWWWmW*
esWaWesWerW itiasWmW

i. *rWisWtoW/rtiasWW W*
  - *teWVito*Wcfiyrg *W* acfiySytyom*W*utorCMg

c. Wash by adding 100µl of ***Wash/Perm*** buffer and spin plates for 5min at 1300rpm at 4˚C, flick off supernatant and vortex plates shortly
d. Wash a second time by adding 200µl of ***Wash/Perm*** buffer and spin plates for 5min at 1300rpm at 4˚C, flick off supernatant and vortex plates shortly
e. Dilute antibody (check dilutions on fridge door!) for intracellular staining in Wash/Perm buffer and add 100µl of antibody solution per well. Do NOT include single stain and unstained controls! Instead, add ***Wash/Perm*** buffer alone to those wells!

   Example:
   2ml Wash/Perm buffer
   20µl IFNg-FITC
   20µl IL-17-PE
   20µl IL-4 APC

   add 100µl per well (enough for 20 wells)

f. Incubate for 30-60min at 4˚C in the fridge
g. Wash by adding 100µl of ***Wash/Perm*** buffer and spin plates for 5min at 1300rpm at 4˚C
h. Flick off supernatant and vortex plates shortly
i. Wash a second time by adding 200µl of ***Wash/Perm*** buffer and spin plates for 5min at 1300rpm at 4˚C
j. Flick off supernatant and vortex plates shortly
k. Resuspend cells in 200µl **FACS buffer** and transfer to 1.2ml library tubes
l. Add additional 200µl FACS buffer to library tubes to achieve a total volume of 400µl.
m. The cells are now ready for acquisition on the FACSCalibur.

Notes:
Cell counting and adjusting the concentration is the exact method. With a little bit of experience one doesn't need to count all the sample and still gets the same quality of data and FACS plots. Here are a few rules of thumb:

Spleen: If one spleen is smashed in resuspended in 10ml, use ca 100-150µl per well.

MLN: If the MLNs from one mouse are liberase digested and resuspended in about 2-3ml, use ca 300µl per well.

LP: The more you can spare the better…