Standard Operating Procedure Lymphocyte/Microglia isolation from mouse CNS for FACS analysis		
DATE: 11.05.11	With changes from Becher's lab Nir's special; AG Waisman	Pages 1/2

1. anesthetize mice (or sacrifice by CO2)
2. perfuse mouse with PBS
3. prepare spinal cord (sc) /brain – transfer into tube filled with PBS (sc into Petri dish with PBS…cut 1cm pieces, prepare like BM(with syringes…)

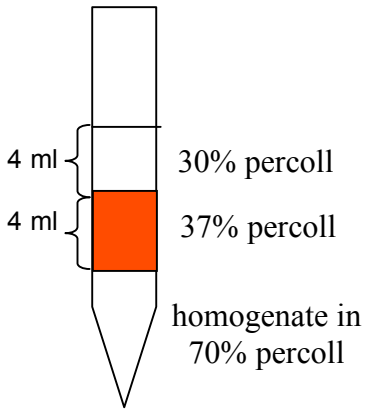
4. dissect tissue to 1-2mm pieces with razor blade (in Petri dish), transfer to 50ml tube, short spin, 'resuspend' in 1-2ml PBS (with **1mg/ml** collagenase + DNase (10-15 units per brain), incubate max. **20min**, 37°C

   note: when pooling brains from few mice less DNase can be used

5. homogenize tissue (add a few ml of PBS) using 5ml syringe with big needle (18-20G) (add some PBS)
6. ↻ 5 min 1500 rpm (=280g) at RT – prepare **NOW** gradients (30%+37% gradient; see below) in 15ml Falcon tube, (**underlay** 4ml of 30% Percoll with 37% Percoll using a spinal cord needle (yellow+long, 20G, 0.9x70mm)

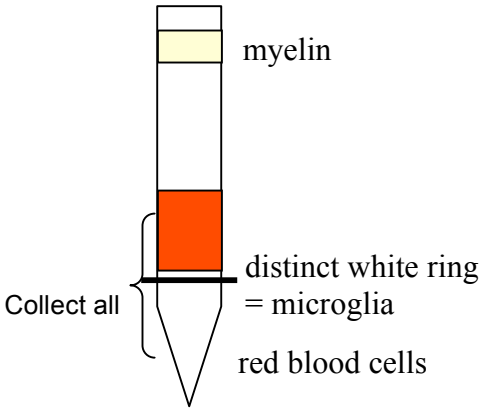
   Isotonic Percoll:
   dilute Percoll (= Sigma original bottle) 10:1 with 10x PBS, (keep stock sterile)
   (= 9parts of Percoll + 1part of 10xPBS)
   - prepare fresh each time
   - this solution is considered as 100% and 30%, 37%, and 70% Percoll are prepared from this freshly prepared solution
   - prepare 30% and 70% Percoll with PBS, 37% with HANKS (red colour!)

7. discard supernatant (be careful, pellet is loose**!**), add 4,5 ml 70% Percoll solution to pellet (final Vol. including pellet ~ 7ml) vortex, homogenize using syringe (no clumps should be left! -otherwise gradient might be destroyed)



8. **underlay** red 37% percoll fraction with 70% percoll/homogenate solution (not more then 6ml ➔ to the bottom of the tube) using a (long) spinal cord infusion needle (yellow+long, 20G, 0.9x70mm) with a 5 ml syringe) – **be extra careful, no bubbles!!!!**

9. ↻ 30 min 2000 rpm **at RT**, **no brakes**!!! (=500g)



10. suck off myelin with water pump
    - note: second ring appears at the beginning of the red fraction, also suck off this ring

11. transfer remaining red fraction PLUS white ring (lymphocytes + microglia) -but NOT the pellet at the very bottom- into **15 ml** Falcon tube, fill up with PBS/EDTA (0.5mM) to 15 ml

12. ↻ 5 min 1500 rpm (=280g) at RT, tilt over, resuspend pellet in left over of PBS, transfer to an eppi tube, spin (small centrifuge, 3500rpm, 5min)
    - now a small pellet should be visible otherwise you did a lot of work for nothing ;-)

13. analyze by FACS

14. take few cells for 'isolation check' (**Fc block!** CD45hi/lo,CD11b) and rest for other stainings (for ic stainings make surface staining on that day - **Fc block!**- and keep fixed cells o/n, make ic staining next day)