

Blood cells separation protocols

Blood Centrifug.	PRP -> platelets Centrifug.	Resuspension platelets	Buffy coat centrifugation	PBMCs centrifugation	Resuspension PBMCs	Reference
500 g 15 min acc 5-6 no brakes	1500g 8-10 min acc 9 brakes 6	Wash with sterile PBS+1 ug/ml PGI2, repellet with 1 ml PBS+PGI2	Dilute 4x with basal RPMI, Layer on Ficoll density gradient (3 ml 1.077+3 ml 1.119) in 15 ml tube. Add 8 ml of diluted blood Centrifugation 700 g 30 min acc 6 no brakes	collect Upper layer MNCs and Middle band PMNs Separately Add 4 volumes of RPMI Centrifuge 700 g 10 min	Resuspend in 1 ml RPMI+0.5% fatty acid free BSA In 1.5 ml tube Centrifuge in picofuge for 30 sec Resuspend in 80 ul RPMI+BSA Add 20 ul magnetic bead labeled antiCD15 Separate by magnetic activated cell sorting (MACS) separator	Jove et al. Wake Forest
16 ml of blood 500 g 10 min acc 9 no brakes	1000g 10 min acc 9 brakes 6	4.5 ml MiR05 or RPMI for intact	Dilute with RPMI , pour on Leucosep tube with Ficoll- Pague 1.077 g/ml, fill up to 50 ml Centrifugation 1000 g 10 min no brakes	Collect PBMCs, dilute with RPMI to 45 ml Centrifuge 200 g 10 min Acc 9 brake 6	4.5 ml MiR05 or RPMI for intact	Florian
35 ml of blood			pour blood diluted with PBS on Leucosep tube with Ficoll- Pague 1.077 g/ml, fill up to 45 ml with PBS Centrifuge 1000 g 10 min RT or 800 g 15 min swinging rotor, no brakes	Collect PBMCs and transfer them in a new 50 ml Falcon tube (without filter). Add 10 ml PBS Centrifuge 250 g 10 min RT	Repeat washing step Centrifuge 250 g 10 min, resuspend in 5 ml PBS	Bioscience Leucosep™ Karabatsiakis et al. 2014 PBS+FCS2%? Freezing in DMSO:FCS- PBS 1:10?
25 ml of blood			pour blood on Leucosep tube with Ficoll- Pague 1.077 g/ml, fill up to 50 ml with PBS Centrifuge 300 g 30 min RT	Collect PBMCs and transfer them in a new 50 ml Falcon tube (without filter). Fill up to 45 ml with PBS Centrifuge 300 g 10 min RT	Wash with 20 ml PBS Centrifuge 300 g 10 min RT resuspend in 1 ml PBS or MiR05	Chiara

200 g 10 min RT		Dilute with Krebs-Henseleit medium without Ca ²⁺ for intact -> respiration Centrifuge 1500 g 10 min RT Resuspend in the same volume of MiR05				Hroudova et al. 2013
6-12 ml of blood in K ₂ EDTA analyze within 3-5 h 300 g 15 min	4600 g 15 min	1-3 ml plasma concentration 1000 – 2500 ml/ml				Sjovall et al. 2013

Respiration of blood cells - protocols

Protocols platelets

Cells + dig 50 ug/ml +M5P5+D1+G5+S10+Omy 2 ug/ml+FCCP 1-3 uM+Rot 0.5 uM+ Ama1.25 ug/ml		Hroudova et al. 2013
P5M5+Dig+D1+G5+S10+Omy 1 uM/ml+FCCP 6 uM+Rot 2 uM+Ama 1 uM/ml+Asc 2 mM+Tm0.5 mM+Azd 10 mM		Sjovall et al. 2013
P5M2+Dig 200 ug/ml+D1+G5+S10+CCCP 1 uM titr +Rot 2 uM+Ama 1.25 ug/ml	200 mil/ml	Florian

Protocols PBMCs (lymphocytes)

G10M2+Dig 15 ug/ml+D2+c10 uM+FCCP 0.8 uM + Rot 0.5 uM	2 mil/ml		Launer et al. 2012
G10M2+Dig 10 ug/ml +D1+ S10+Omy 2 ug/ml+CCCP 1.5 uM+Rot 5 uM+ Ama 2 uM		lymphoblasts	Lee et al. 2012
M3P10+G10+S10+GP10+D1+5-200 nM Omy+150-200 nM FCCP-> 0 O2+reox+ Ama0.25 uM + Asc 2 mM+ TMPD 0.6 mM+ KCN 0.5 mM		0.6 mg protein	Pecina et al. 2014
P5M2+Dig 20 ug/ml+D1+G5+S10+CCCP 1 uM titr+Rot 2 uM+Ama 1.25 ug/ml	3 mil/ml		Florian
P5M2+Dig 20 ug/ml+D1+G5+S10+CCCP+Rot 2 uM + Ama 1.25 ug/ml	2-3 mil/ml		Chiara
OcXM2+D2+P5+c+G10+S10+CCCP+S50+Rot+GP+Ama+Asc+Tm+Azd Red should be adjusted, if S50 stimulatory, add before CCCP		MiR05Cr	Proposed protocol for Wake Forest

Protocol intact cells

Routine+Omy 1 ug/ml+FCCP (6 uM in PBS, 100 uM in plasma)+Rot 2 uM+Ama 1ug/ml	platelets	Plasma PBS	Sjovall et al. 2013
Routine+Omy 5 ug/ml+FCCP (0.5 uM)+Rot 0.5 uM+Ama 2.5 ug/ml	PBMCs	MiR05	Karabatsiakos et al. 2014
Routine+Omy+CCCP 9 uM+Rot 2 uM+Ama1.25 uM	PBMCs	MiR05	Chiara

Brief conclusions on blood cells:

- Work with fresh blood if possible, if not, set the same conditions for all samples.
- Whole blood could stay at RT up to 24 h, after that, decline in respiration.
- Work in RT – all centrifugation steps, media.

Chiara: PBS works well with resuspension and transport of PBMCs, cells transported in MiR05 for longer than 20 min suffer!
PBMCs for respiration > 1 mil/ml, 2 or 3 mil/ml no difference in flux per cell

Florian: Platelets for respiration: 200 mil/ml
PBMCs for respiration 3 mil/ml

Suggestion of Alexander: make hemogram for blood cell content!
Suggestion of Dan Tyrrel: use PGI2 against coagulation of platelets.

Suggested protocol for both cell types:

Check:

A: Oc0.5+D1+M0.05+M0.1+M0.2+M0.5+M1+M2+Oc0.5+P+c+G+D2.5+S10+FCCP+S50+Rot+GP+Ama+Asc+Tm+Azd

If S50 in ETS stimulatory, add after G S50!

B: Oc0.5+Mx+D5+Oc0.5+M2+P+c+G+S50+D2.5+FCCP+Rot+GP+Ama+Asc+Tm+Azd

Final protocol: Oc**XM**X+D5+M2+P5+c+G10+S10+D7.5+CCCP+S50+Rot+GP+Ama+Asc+Tm+Azd

Protocols respiration - skeletal muscle

M2+Oc0.2+D2.5+G10+S10+c10uM+D5+FCCP 1.25 uM+Rot 0.5 uM+Ama 2.5 uM D5 necessary, MiR06	2.8 mg w.w.	Human vastus lateralis	Pesta et al. AmJPRICP 2011
Oc XM 2+D5+P5+c+G10+S10+D7.5+CCCP+S50+Rot+GP+Ama+Asc+Tm+Azd Red should be adjusted, if S50 stimulatory, add before CCCP		MiR06Cr	Proposed protocol for Wake Forest

Type IIb skeletal muscle mitochondria – greater capacity for **GP oxidation!** (Jackman and Willis, American Physiology Society 1996.)

Final protocol: Oc**XM**X+D5+M2+P5+c+G10+S10+D7.5+CCCP+S50+Rot+GP+Ama+Asc+Tm+Azd

Proposed protocols to check in MiR06Cr 13.1.2016: mouse soleus

1. **A:** Oc0.5+D5+M0.05+M0.1+M0.2+M0.5+M1+M2+Oc0.5+P+c+G+S10+D7.5+FCCP+S50+Rot+GP+ Ama+Asc+Tm+Azd

B: Oc0.5+D5+M0.05+M0.1+M0.2+M0.5+M1+M2+Oc0.5+P+c+G+D7.5+S10+FCCP+S50+Rot+ GP+Ama+Asc+Tm+Azd **1s**

If S50 in ETS stimulatory, add after G S50!

2. **A:** Oc0.5+D5+Mx+Oc0.5+M2+P+c+G+S50+D7.5+FCCP+ Rot+GP+Ama+Asc+Tm+Azd

B: Oc0.5+D5+Mx+Oc0.5+M2+P+c+G+D7.5+S50+FCCP+ Rot+GP+Ama+Asc+Tm+Azd **2s**

3. **A:** PM2+D5+c+Rot+Oc **1s**

B: Oc0.5+M2+D5+P+c+Rot