

Materials List for:

# *In Vitro* and *In Vivo* Assessment of T, B and Myeloid Cells Suppressive Activity and Humoral Responses from Transplant Recipients

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## Materials

Name	Company	Catalog Number	Comments
<b>animals</b>			
LEW.1W and LEW.1A rats	Janvier Labs, France		8 weeks old,
BN third party donor rats	Janvier Labs, France		8 weeks old,
<b>name</b>	<b>company</b>	<b>catalogue number</b>	<b>comments</b>
<b>reagents</b>			
AdCD40lg	Viral Vector Core, INSERM UMR 1089, Nantes, France		home made plasmids
IL34-AAV	Viral Vector Core, INSERM UMR 1089, Nantes, France		home made plasmids
FGL2-AAV	Viral Vector Core, INSERM UMR 1089, Nantes, France		home made plasmids
anti-TCRab	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	R7/3 clone	Home made culture, purification and fluororophore coupling
anti-CD25	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX39 clone	Home made culture, purification and fluororophore coupling
anti-CD8	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX8 clone	Home made culture, purification and fluororophore coupling
anti-CD45RA	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX33 clone	Home made culture, purification and fluororophore coupling
anti-CD161	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	3.2.3 clone	Home made culture, purification and fluororophore coupling
anti-CD11b/c	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX42 clone	Home made culture, purification and fluororophore coupling
anti-TCRgd	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	V65 clone	Home made culture, purification and fluororophore coupling
anti-CD45RC	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX22 clone	Home made culture, purification and fluororophore coupling
anti-CD4	Hybridoma from European Collection of Cell Culture, Salisbury, U.K	OX35 clone	Home made culture, purification and fluororophore coupling
anti-CD45R	BD Biosciences, Mountain View, CA	#554881, His24 clone	

anti-rat IgG-FITC	Jackson ImmunoResearch Laboratories, INC, Baltimore, USA	#112-096-071	
anti-rat IgG1	Serotec	#MCA 194	
anti-rat IgG2a	Serotec	#MCA 278	
anti-rat IgG2b	Serotec	#MCA 195	
anti-rat IgM-FITC	Jackson ImmunoResearch Laboratories, INC, Baltimore, USA	#115-095-164	
streptavidin HRP	BD Biosciences, Mountain View, CA	#554066	
KLH	Sigma Aldrich, St. Louis, USA	#9013-72-3	
PBS 1X	Thermo Fisher Scientific Inc, USA		Phosphate Buffer Solution without calcium and magnesium,
Tween 20	Sigma, Saint-Louis, USA	#9005-64-5	
TMB substrate reagent kit	BD Biosciences, Mountain View, CA	#555214	
CellTrace™ CFSE cell proliferation kit	Thermo Fisher Scientific Inc, USA	#C34554	
RPMI 1640 medium 1X	Thermo Fisher Scientific Inc, USA	#31870-025	
penicilline streptomycine	Thermo Fisher Scientific Inc, USA	#15140-122	
Hepes Buffer	Thermo Fisher Scientific Inc, USA	#15630-056	
non essential amino acids	Thermo Fisher Scientific Inc, USA	#11140-035	
Sodium pyruvate	Thermo Fisher Scientific Inc, USA	#11360-039	
2 beta mercaptoethanol	Sigma, Saint-Louis, USA	#M3148	
Cell Proliferation Dye eFluor® 450 Cell	Thermo Fisher Scientific Inc, USA	#65-0842-85	
Glutamine	Sigma, Saint-Louis, USA	#G3126	
DAPI	Thermo Fisher Scientific Inc, USA	#D1306	
Collagenase D	Roche Diagnostics, Germany	#11088882001	
EDTA	Sigma, Saint-Louis, USA	#E5134	
NaCl 0.9%	Fresenius Kabi	#B230561	
Magnetic dynabeads	Dynal, Invitrogen	#11033	Goat anti-mouse IgG
One Comp eBeads	Ebiosciences, San Diego, USA	#01-1111-42	
Betadine	Refer to the institutional guidelines		
Isoflurane	Refer to the institutional guidelines		
Naplbuphine	Refer to the institutional guidelines		
Terramycine	Refer to the institutional guidelines		
Buprenorphine	Refer to the institutional guidelines		
Meloxicam	Refer to the institutional guidelines		
Complete Freund's adjuvant			
Rompun	Refer to the institutional guidelines		
Ringer lactate	Refer to the institutional guidelines		
Ketamine	Refer to the institutional guidelines		
Red blood cell lysis solution			Dilute 8,29g NH4Cl (Sigma, Saint-Louis, USA A-9434), 1g KHCO3 (Prolabo 26 733.292) and 37.2mg EDTA (Sigma, Saint-Louis, USA E5134) in 800ml H2O. Adjust pH to 7.2-7.4 and complete to 1L with H2O.

Collagenase D			Dilute 1g collagenase in 500 ml RPMI-1640 + 5 ml Hepes + 2% FCS
PBS-FCS (2%)-EDTA (0.5%)			Add 5 mL EDTA 0,1M (Sigma, Saint-Louis, USA E5134) and 20ml FCS to 1ml PBS 1X
CFSE (Vybrant CFDA SE Cell Tracer Kit Invitrogen)			Dilute 50µg (=1 vial) of CFDA SE (component A) in 90µl DMSO (component B) solution to obtain a 10mM stock solution. Then, dilute stock solution at 1/20 000 in PBS 1X to obtain a 0.5µM solution
complete medium for coculture			500ml complete RPMI-1640 medium with 5 ml Penicillin (80 unit/ml)-Streptomycin (80 mg/ml), 5 ml L-Glutamine, 5 ml Non Essential Amino Acids (100X), 5ml Pyruvate Sodium (100mM), 5 ml HEPES buffer (1M), 2.5 ml b mercaptoethanol (7 ml of 2-bmercaptoethanol stock diluted in 10 ml RPMI), 10% FCS
<b>name</b>	<b>company</b>	<b>catalog number</b>	<b>comments</b>
<b>equipments</b>			
falcon 50ml	BD Biosciences, Mountain View, CA	#227261	
falcon 15ml	BD Biosciences, Mountain View, CA	#188271	
sieve			
Corning plastic culture dishes	VWR, Pessac	#391-0439	
100µm and 60µm tissue filters	Sefar NITEX, Heiden, Switzerland	#03-100/44 and #03-60/35	
96 wells U bottom plates for coculture	Falcon U-bottom Tissue Culture plate, sterile, Corning	#353077	
96 wells V bottom plates for FACS staining	ThermoScientifique, Danemark	#249570	
96 wells flat bottom ELISA plates	Nunc Maxisorb		
seringue for spleen crush	BD Biosciences, Mountain View, CA	#309649	
ELISA reader	SPARK 10M, Tecan, Switzerland	SPARK 10M, Tecan, Switzerland	
centrifuge			
bain marie			
X rays irradiator	Lincolshire, England	Faxitron CP160	
solar agitator			
FACS Canto II	BD Biosciences, Mountain View, CA		
FACS Aria II	BD Biosciences, Mountain View, CA		
magnet	Thermo Fisher Scientific Inc, USA	12302D	