

Materials List for

Lentiviral Vector Preparation for Efficient Gene and MicroRNA Modulation of Peritoneal Cavity Tissue-Resident Macrophages *In Vivo* in Mice

Mark Gurney¹, Luke C. Davies^{1,2}, Ruth E. Jones¹, Valentina M. Bart¹, Robert H. Jenkins¹, Paul Brennan¹, Philip R. Taylor^{1,3}, Magdalena A. Czubala¹

Citation		
Gurney, M., Davies, L.C., Jones,	R.E., Bart, V.M., Jenkins, R.H., Brennan, P., Taylor, P.R.,	
Czubala, M.A. Lentiviral Vector Preparation for Efficient Gene and MicroRNA Modulation of		
Peritoneal Cavity Tissue-Resident Macrophages In Vivo in Mice. J. Vis. Exp. (204), e64926,		
doi:10.3791/64926 (2024).		
DOI	URL	
10.3791/64926	jove.com/video/64926	
	Gurney, M., Davies, L.C., Jones, Czubala, M.A. Lentiviral Vector F Peritoneal Cavity Tissue-Resider doi:10.3791/64926 (2024).	

Materials

Name	Company	Catalog Number	Comments
0.05% Trypsin-EDTA (1x) (Trypsin 500 mg/L or 0.02 mM)	Thermo Fisher Scientific	25300054	
0.22 µm sterile millex GP filter	Merck	SLGS033SS	
0.45 µm sterile millex GP filter	Merck	SLHP033RS	
0.5 mL U-100 insulin syringe with needle, 0.33 mm x 12.7 mm (29 G)	BD	324892	
1 Litre Sharps Container	N/A	N/A	
2.4G2 antibody (TruStain FcX antimouse CD16/32)	Biolegend	101320	
40 μm strainer	Thermo Fisher Scientific	22363547	
AimV medium (research grade), AlbuMax Supplement	Thermo Fisher Scientific	31035025	
Blocking buffer	prepared in house		
Brewer thioglycolate medium	Sigma-Aldrich	B2551	4% stock solution prepared in water, autoclaved and kept frozen.
CD11b	Biolegend	101222	Refer to Table 1 for the dilution and concentration
CD11b	BD	550993	Refer to Table 1 for the dilution and concentration
CD11c	Biolegend	117317	Refer to Table 1 for the dilution and concentration
CD11c	Biolegend	117333	Refer to Table 1 for the dilution and concentration
CD19	BD	560375	Refer to Table 1 for the dilution and concentration
CD19	Biolegend	152410	Refer to Table 1 for the dilution and concentration

¹Systems Immunity Research Institute, Division of Infection and Immunity, Cardiff University ²Biomedical Sciences Unit, Faculty of Medicine, Health and Life Science, Swansea University ³UK Dementia Research Institute



	,		
CD226	Biolegend	128808	Refer to Table 1 for the dilution and concentration
CD3e	BD	560527	Refer to Table 1 for the dilution and concentration
CD3e	Biolegend	152313	Refer to Table 1 for the dilution and concentration
CD4	Biolegend	100412	Refer to Table 1 for the dilution and concentration
CD73	eBioscience	16-0731-82	Refer to Table 1 for the dilution and concentration
CD8a	eBioscience	48-0081-82	Refer to Table 1 for the dilution and concentration
Cell culture flask (T175 fask, 175 cm ² , 550 mL)	Greiner Bio One	658175	
Centrifuge tubes, conical bottom tubes 25 mm x 89 mm	Beckman Coulter	358126	
Centrifuges	Beckman Coulter		Ultracentrifuge and TC centrifuge
Collagenase type IV	Sigma-Aldrich	C5138	
Conical centrifuge tubes (15 mL and 50 mL)	Greiner Bio One	11512303 & 11849650	
Cryotubes	Greiner Bio One	123277	or cryotubes
DMEM medium (1x) + 4.5g/L D- glucose, 400 µM L-glutamine	Thermo Fisher Scientific	41965-062	
Dnase I	Sigma-Aldrich	11284932001	
Effectene transfection reagent	Qiagen	301425	
F4/80	Biolegend	123123	Refer to Table 1 for the dilution and concentration
F4/80	Biolegend	123133	Refer to Table 1 for the dilution and concentration
F4/80	Biolegend	123147	Refer to Table 1 for the dilution and concentration
FceR1	eBioscience	48-5898-80	Refer to Table 1 for the dilution and concentration
Fetal calf serum (FCS)	Thermo Fisher Scientific	10270-106	heat inactivated for 30 min at 56 °C and sterile filtered through 0.22 µm filter
Flow cytometer	Thermo Fisher Scientific	Attune NxT	
Flow cytometry (FACS) buffer	prepared in house		
Fluorescent tissue culture microscope	Thermo Fisher Scientific	EVOS FL	
Forceps	N/A	N/A	User preference
Hank's balanced salt solution (HBSS)	Gibco, Life Technologies	14175-053	
HEK293T cell line			grown for at least a week prior transfection. Mycoplasma free
HIV-1 Core antigen	Beckman Coulter	6604667	
Hyaluronidase	Sigma-Aldrich	H3506	
Hydrex surgical scrub, chlorhexiding gluconate 4% w/v skin cleanser	Ecolab	3037170	
I-A/I-E	Biolegend	107625	Refer to Table 1 for the dilution and concentration
ICAM1	Becton Dickinson	554970	Refer to Table 1 for the dilution and concentration
Jurkat T cell line			grown for at least a week prior use. Mycoplasma free



LIVE/DEAD fixable near-IR dead cell	Thermo Fisher Scientific	L34975	
stain kit	Thermo i loner delentino	204070	
Ly6G	Biolegend	127615	Refer to Table 1 for the dilution and concentration
Mice			here used C57BL/6 females, aged 8-12 weeks (Charles Rivers), unless specified differently
Microcapillary pipettes (volume range 0.5-1,000 μ L)	Fisher Scientific & Starlab	11963466 & 11943466 & 11973466 & S1111-3700	
NK1.1	Biolegend	108724	Refer to Table 1 for the dilution and concentration
Paraformaldehyde	Sigma-Aldrich	P6148-500G	prepared to 2% w/v in PBS
pCMV-ΔR8.91 packaging plasmid		Zuffrey, R., et al. 1997	encodes Gag-Pol HIV protein driven by cytomegalovirus promoter. Ampicilin resistance.
Penicillin/Streptomycin (100x, 10,000 U/mL)	Thermo Fisher Scientific	15140122	
Petri dish	Greiner Bio One	664160	
pHR'SIN-cPPT-SEW plasmid		Rosas, M. et al. 2014	modified for shRNA and miR expression studies. Encodes EGFP marker downstream SFFV promoter and upstream of the Woodchuck hepatitiv virus enhancer. Ampicilin resistance.
pMD2.G plasmid		Naldini, L. et al. 1996	encodes vesicular stomatis virus g-glycoprotein (VSV-G) envelope. Ampicilin resistance.
Rat IgG1, κ isotype control	Becton Dickinson	550617	Refer to Table 1 for the dilution and concentration
Rat serum	Sigma-Aldrich	R9759-10ML	
Red blood ACK lysis buffer	prepared in house		
RPMI 1640 medium (1x) + 400 uM L- glutamine	Thermo Fisher Scientific	21875-091	
Saponin	Sigma-Aldrich	S4521	
SiglecF	BD	562681	Refer to Table 1 for the dilution and concentration
Sodium hypochlorite Tablets (bleach, 2,000 ppm)	Guest Medical	H8818	
Sterile 24-well cell culture plate	Greiner Bio One	662160	
Sterile Dulbecco's PBS (DPBS) (1x) Mg ⁺⁺ and Ca ²⁺ - free	Thermo Fisher Scientific	14190144	
Sterile EDTA	Thermo Fisher Scientific	15575020	
Sterile VWR disposable transfer pipets (23.0 mL, 30 cm)	VWR	612-4515	
Sucrose	Thermo Fisher Scientific	15503022	
surgical scissors	N/A	N/A	User preference
Syringes (50 mL and 1 0mL)	Fisher Scientific	10084450 & 768160	
Tim4	Biolegend	130007	Refer to Table 1 for the dilution and concentration
U-bottom 96-well cell culture plate	Greiner Bio One	650180	