

Materials List for

Chemical Conjugation of a Purified DEC-205-Directed Antibody with Full-Length Protein for Targeting Mouse Dendritic Cells *In Vitro* and *In Vivo*

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Materials

Name	Company	Catalog Number	Comments
antibody buffer 2 %			2 % (w/v) Slim-Fast Chocolate powder in TBS-T
antibody buffer 5 %			5 % milk powder (w/v) in TBS-T
blocking buffer (ELISA)			10 % FBS in PBS
blocking buffer 4 %			4 % (w/v) Slim-Fast Chocolate powder in TBS-T
blocking buffer 10 %			10 % milk powder (w/v) in TBS-T
cell culture flask T25	Greiner Bio-One	690175	we use standard CELLSTAR filter cap cell culture flasks; alternatively use suspension culture flask (690195)
cell culture flask T75	Greiner Bio-One	658175	we use standard CELLSTAR filter cap cell culture flasks; alternatively use suspension culture flask (658195)
cell culture flask T175	Greiner Bio-One	661175	we use standard CELLSTAR filter cap cell culture flasks; alternatively use suspension culture flask (661195)
centrifugal concentrator MWCO 10 kDa	Sartorius	VS2001	Vivaspin 20 centrifugal concentrator
centrifugal protein concentrator MWCO 100 kDa, 5 - 20 ml	Thermo Fisher Scientific	88532	Pierce Protein Concentrator, PES 5 -20 ml; we use the Pierce Concentrator 150K MWCO 20mL

			(catalog number 89921), which is however no longer available
centrifuge bottles	Nalgene	525-2314	PPCO (polypropylene copolymer) with PP (polypropylene) screw closure, 500 ml; obtained from VWR, Germany
coating buffer (ELISA)			0.1 M sodium bicarbonate (NaHCO ₃) in H ₂ O (pH 9.6)
desalting columns MWCO 7 kDa	Thermo Fisher Scientific	89891	Thermo Scientific Zeba Spin Desalting Columns, 7K MWCO, 5 mL
detection reagent ELISA (HRPO substrate)	Sigma-Aldrich/Merck	T8665-100ML	3,3',5,5'-Tetramethylbenzidine (TMB) liquid substrate system
detection reagent western blot (HRPO substrate)	Roche/Merck	12 015 200 01	Lumi-Light Western Blotting Substrate (Roche)
dialysis tubing MWCO 12 - 14 kDa	SERVA Electrophoresis	44110	Visking dialysis tubing, 16 mm diameter
ELISA 96-well plate	Thermo Fisher Scientific	442404	MaxiSorp Nunc-Immuno Plate
fetal calf serum	PAN-BIOtech	P40-47500	FBS Good forte
ISF-1 medium	Biochrom/bioswisstec	F 9061-01	
milk powder	Carl Roth	T145.2	powdered milk, blotting grade, low in fat; alternatively we have also used conventional skimmed milk powder from the supermarket
NLDC-145 hybridoma	ATCC	HB-290	if not already at hand, the hybridoma cells can be acquired from ATCC
non-reducing SDS sample buffer 4 x			for 12 ml: 4 ml of 10 % SDS, 600 µl 0.5 M Tris-HCl (ph 6.8), 3.3 ml sterile H ₂ O, 4 ml glycerine, 100 µl of 5 % Bromphenol Blue
ovalbumin	Hyglos (via BioVendor)	321000	EndoGrade OVA ultrapure with <0.1 EU/mg
Penicillin/Streptomycin	Thermo Fisher Scientific	15140122	Gibco Penicillin/Streptomycin 10.000 U/ml; alternatively Gibco Penicillin/Streptomycin 5.000 U/ml (15070-063) can be used
PETG polyethylene terephthalate glycol cell culture roller bottles	Nunc In Vitro	734-2394	standard PDL-coated, vented (1.2X), 1050 cm ² , 100 - 500 ml volume; obtained from VWR, Germany
pH indicator strips	Merck	109535	pH indicator strips 0-14
polyclonal goat arat-IgG(H+L)-HRPO (western blot)	Jackson ImmunoResearch	112-035-062	obtained from Dianova, Germany; used at 1:5000 for western blot
polyclonal goat arat-IgG+IgM-HRPO antibody (ELISA)	Jackson ImmunoResearch	112-035-068	obtained from Dianova, Germany; used at 1:2000 for ELISA
polyclonal goat arabbit-IgG-HRPO (western blot)	Jackson ImmunoResearch	111-035-045	obtained from Dianova, Germany; used at 1:2000 for western blot
polyclonal rabbit αOVA (ELISA)	Abcam	ab181688	used at 3 ng/µl
polyclonal rabbit αOVA antibody (western blot)	OriGene	R1101	used at 1:3,000 for western blot
Protein G Sepharose column	Merck/Millipore	P3296	5 ml Protein G Sepharose, Fast Flow are packed onto an empty column PD-10 (Merck, GE 17-0435-01)
protein standard	Thermo Fisher Scientific	26616	PageRuler Prestained Protein ladder 10 - 180 kDa
PVDF (polyvinylidene difluoride) membrane	Merck/Millipore	IPVH00010	immobilon-P PVDF (polyvinylidene difluoride) membrane
rubber plug	Omnilab	5230217	DEUTSCH & NEUMANN rubber stoppers (lower Φ 17 mm; upper Φ 22 mm)

silicone tube	Omnilab	5430925	DEUTSCH & NEUMANN (inside Φ 1 mm; outer Φ 3 mm)
Slim-Fast			we have used regular Slim-Fast Chocolate freely available at the pharmacy as in this western blot approach it yielded better results than milk powder
stopping solution (ELISA)			1M H ₂ SO ₄
sulfo-SMCC	Thermo Fisher Scientific	22322	Pierce Sulfo-SMCC Cross-Linker; alternatively use catalog number A39268 (10 x 2 mg)
syringe filter unit 0.22 μ m	Merck/Millipore	SLGV033RS	Millex-GV Syringe Filter Unit, 0.22 μ m, PVDF, 33 mm, gamma sterilized
syringe 10 ml	Omnilab		Disposable syringes Injekt® Solo B.Braun
Sterican® cannulas	B. Braun		Sterican® G 20 x 1 1/2"; 0.90 x 40 mm; yellow
TBS-T			Tris-buffered saline containing 0.1 % (v/v) Tween 20
TCEP-HCl	Thermo Fisher Scientific	A35349	
tubing connector	Omnilab		Kleinfeld miniature tubing connectors for silicone tube