Materials List for:

Generation of Discriminative Human Monoclonal Antibodies from Rare Antigen-specific B Cells Circulating in Blood

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Materials

Name	Company	Catalog Number	Comments
HEK 293A cell line	Thermo Fisher scientific	R70507	
DMEM (1X) Dulbecco's Modified Eagle Medium	Gibco by life technologies	21969-035	(+) 4,5g/L D-Glucose 0,11g/L Sodium Pyruvate (-) L-Glutmine
RPMI medium1640 (1X)	Gibco by life technologies	31870-025	
Bovine Serum Albumine (BSA)	PAA	K45-001	
Nutridoma-SP	Roche	11011375001	100X Conc
PBS-Phosphate Buffered Saline (10X) pH 7,4	Ambion	AM9624	
EDTA (Ethylenediaminetetraacetic acid) 0,5M pH=8	Invitrogen by Life Technologies	15575-020	
Fetal Bovine serum (FBS)	Dominique Dutscher	S1810-500	
Ficoll - lymphocytes separation medium	EuroBio	CMSMSL01-01	density 1,0777+/-0,001
streptavidin R-phycoerythrin conjugate	Invitrogen by Life Technologies	S21388	premiun grade 1mg/ml contains 5mM sodium azide
Streptavidin, allophycocyanin conjugate	Invitrogen by thermoFisher scientific	S32362	1mg/ml 2mM azide premium grade
Brilliant violet 421 streptavidin	Biolegend	405225	conc : 0,5mg/ml
Anti-PE conjugated magnetic MicroBeads	Miltenyi Biotec	130-048-801	
Anti-APC conjugated magnetic MicroBeads	Miltenyi Biotec	130-090-855	
MidiMACs or QuadroMACS separotor	Miltenyi Biotec	130-042-302/130-090-976	
LS Columns	Miltenyi Biotec	130-042-401	
CD3 BV510 BD horizon	BD Pharmingen / BD Biosciences	563109	Used dilution 1:20
CD19 FITC	BD Pharmingen / BD Biosciences	345788	Used dilution 1:20
CD14 PerCPCy5.5	BD Pharmingen / BD Biosciences	561116	Used dilution 1:50
CD16 PerCPCy5.5	BD Pharmingen / BD Biosciences	338440	Used dilution 1:50
7AAD	BD Pharmingen / BD Biosciences	51-68981E (559925)	Used dilution 1:1000
FACS ARIA III Cell Sorter Cytometer	BD Biosciences		
8-strip PCR tubes	Axygen	321-10-061	
Racks for 96 microtubes	Dominique Dutscher	45476	

RNAseOUT Ribonuclease Inhibitor (recombinant)	Invitrogen by thermoFisher scientific	10777-019	qty:5000U (40U/ul)
Distilled Water Dnase/Rnase Free	Gibco	10977-035	
Oligod(T)18 mRNA Primer	New England BioLabs	S1316S	5.0 A260unit
Random hexamers	Invitrogen by thermoFisher scientific	N8080127	qty : 50uM, 5nmoles
Superscript III Reverse transcriptase	Invitrogen by thermoFisher scientific	18080-044	qty : 10000U (200U/ul)
GoTaq G2 Flexi DNA polymerase	Promega	M7805	
dNTP Set, Molecular biology grade	Thermo Scientific	R0182	4*100umol
5LVH1	Eurofins	ACAGGTGCCCACT CCCAGGTGCAG	First round of PCR - Amplification of heavy chains - Outer primers - Forward Prmers
5LVH3	Eurofins	AAGGTGTCCAGTG TGARGTGCAG	First round of PCR - Amplification of heavy chains - Outer primers - Forward Prmers
5LVL4_6	Eurofins	CCCAGATGGGTCC TGTCCCAGGTGCAG	First round of PCR - Amplification of heavy chains - Outer primers - Forward Prmers
5LVH5	Eurofins	CAAGGAGTCTGTT CCGAGGTGCAG	First round of PCR - Amplification of heavy chains - Outer primers - Forward Prmers
3HuIgG_const_anti	Eurofins	TCTTGTCCACCTT GGTGTTGCT	First round of PCR - Amplification of heavy chains - Outer primers -Reverse primers for human Ig-Bacteria PCR screening
3CuCH1	Eurofins	GGGAATTCTCACA GGAGACGA	First round of PCR - Amplification of heavy chains - Outer primers - Reverse primers for human Ig
5AgeIVH1_5_7	Eurofins	CTGCAACCGGTGTACATTCC GAGGTGCAGCTGGTGCAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH3	Eurofins	CTGCAACCGGTGTACATTCT GAGGTGCAGCTGGTGGAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH3_23	Eurofins	CTGCAACCGGTGTACATTCT GAGGTGCAGCTGTTGGAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH4	Eurofins	CTGCAACCGGTGTACATTCC CAGGTGCAGCTGCAGGAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH4_34	Eurofins	CTGCAACCGGTGTACATTCC CAGGTGCAGCTACAGCAGTG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH1_18	Eurofins	CTGCAACCGGTGTACATTCC CAGGTTCAGCTGGTGCAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH1_24	Eurofins	CTGCAACCGGTGTACATTCC CAGGTCCAGCTGGTACAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH3__9_30_33	Eurofins	CTGCAACCGGTGTACATTCT GAAGTGCAGCTGGTGGAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers
5AgeIVH6_1	Eurofins	CTGCAACCGGTGTACATTCC CAGGTACAGCTGCAGCAG	Second round of PCR - Amplification of heavy chains - Inner primers -Forward primers

3SalIJH1_2_4_5	Eurofins	TGCGAAGTCGACG CTGAGGAGACGGTGACCAG	Second round of PCR - Amplification of heavy chains - Inner primers -Reverse primers
3SalIJH3	Eurofins	TGCGAAGTCGACG CTGAAGAGACGGTGACCATTG	Second round of PCR - Amplification of heavy chains - Inner primers -Reverse primers
3SalIJH6	Eurofins	TGCGAAGTCGACG CTGAGGAGACGGTGACCGTG	Second round of PCR - Amplification of heavy chains - Inner primers -Reverse primers
5'LVk1_2	Eurofins	ATGAGGSTCCCYG CTCAGCTGCTGG	First round of PCR - Amplification of light chains k - Outer primers - Forward primers
5'LVk3	Eurofins	CTCTTCCTCCTGC TACTCTGGCTCCCAG	First round of PCR - Amplification of light chains k - Outer primers - Forward primers
5'LVk4	Eurofins	ATTTCTCTGTTGC TCTGGATCTCTG	First round of PCR - Amplification of light chains k - Outer primers - Forward primers
3'Ck543_566	Eurofins	GTTTCTCGTAGTC TGCTTTGCTCA	First round of PCR - Amplification of light chains k - Outer primers - Reverse primers- Bacteria PCR screening
5'AgeIVk1	Eurofins	CTGCAACCGGTGTACATTCT GACATCCAGATGACCCAGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeIVk1_9_1–13	Eurofins	TTGTGCTGCAACCGGTGTAC ATTCAGACATCCAGTTGACCCAGTCT	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeIVk1D_43_1_8	Eurofins	CTGCAACCGGTGTACATTGT GCCATCCGGATGACCCAGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeIVk2	Eurofins	CTGCAACCGGTGTACATGGG GATATTGTGATGACCCAGAC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeIVk2_28_2_30	Eurofins	CTGCAACCGGTGTACATGGG GATATTGTGATGACTCAGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeVk3_11_3D_11	Eurofins	TTGTGCTGCAACCGGTGTAC ATTCAGAAATTGTGTTGACACAGTCT	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeVk3_15_3D_15	Eurofins	CTGCAACCGGTGTACATTCA GAAATAGTGATGACGCAGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeVk3_20_3D_20	Eurofins	TTGTGCTGCAACCGGTGTAC ATTCAGAAATTGTGTTGACGCAGTCT	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
5'AgeVk4_1	Eurofins	CTGCAACCGGTGTACATTCG GACATCGTGATGACCCAGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
3'BsiWIJk1_2_4	Eurofins	GCCACCGTACGTT TGATYTCCACCTTGGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
3'BsiWIJk3	Eurofins	GCCACCGTACGTT TGATATCCACTTTGGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers
3'BsiWIJk5	Eurofins	GCCACCGTACGTT TAATCTCCAGTCGTGTC	Second round of PCR - Amplification of light chains k - Inner primers -Forward primers

5'LVl1	Eurofins	GGTCCTGGGCCCA GTCTGTGCTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'LVl2	Eurofins	GGTCCTGGGCCCA GTCTGCCCTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'LVl3	Eurofins	GCTCTGTGACCTC CTATGAGCTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'LVl4_5	Eurofins	GGTCTCTCTCSCA GCYTGTGCTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'LVl6	Eurofins	GTTCTTGGGCCAA TTTTATGCTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'LVl7	Eurofins	GGTCCAATTCYCA GGCTGTGGTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5LVl8	Eurofins	GAGTGGATTCTCA GACTGTGGTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
3'Cl	Eurofins	CACCAGTGTGGCC TTGTTGGCTTG	First round of PCR - Amplification of light chains λ - Outer primers - Forward primers
5'AgeIVl1	Eurofins	CTGCTACCGGTTCCTGGGCC CAGTCTGTGCTGACKCAG	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
5'AgeIVl2	Eurofins	CTGCTACCGGTTCCTGGGCC CAGTCTGCCCTGACTCAG	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
5'AgeIVl3	Eurofins	CTGCTACCGGTTCTGTGACC TCCTATGAGCTGACWCAG	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
5'AgeIVl4_5	Eurofins	CTGCTACCGGTTCTCTCTCS CAGCYTGTGCTGACTCA	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
5'AgeIVl6	Eurofins	CTGCTACCGGTTCTTGGGCC AATTTTATGCTGACTCAG	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
5'AgeIVl8	Eurofins	CTGCTACCGGTTCCAATTCY CAGRCTGTGGTGACYCAG	Second round of PCR - Amplification of light chains λ - Inner primers -forward primers
3'XhoICl	Eurofins	CTCCTCACTCGAG GGYGGGAACAGAGTG	Second round of PCR - Amplification of light chains λ - Inner primers -Reverse primers - Bacteria PCR screening
Ab-vec-sense	Eurofins	GCTTCGTTAGAAC GCGGCTAC	Bacteria PCR screening
QA Agarose-TM, Molecular Biology Grade	MP Bio	AGAH0500	
NucleoFast 96 PCR Plate	Macherey Nagel	743.100.100	
Enzyme Age I HF	New England Biolabs	R3552L	20000U/ml
Enzyme SalI HF	New England Biolabs	R3138L	20000U/ml
Enzyme Xho I	New England Biolabs	R0146L	20000U/ml
Enzyme BSIWI	New England Biolabs	R0553L	10000U/ml
HCg1 (Genbank accession number FJ475055)			

LCk (Genbank accession number FJ475056)			
LCl (Genbank accession number FJ517647)			
T4 DNA ligase	Invitrogen by thermoFisher scientific	15224.017	100U (1U/ul)
2X YT medium	Sigma Aldrich	Y1003-500ML	
Ampicillin	Sigma Aldrich	10835242001	
LB (Luria Bertani) Broth (Lennox)	Sigma Aldrich	L3022-250G	
Nucleospin Plasmid DNA, RNA and protein purification	Macherey Nagel	740588.250	
Jet PEI DNA transfection reagent	PolyPlus	101-40	
Flat bottom96-well plate	Falcon	353072	
V-bottom 96-well plate	Nunc/Thermofisher	055142	
Nunc easy 175 cm2 flasks	Nunc/Thermofisher	12-562-000	
ELISA/ELISPOT coating buffer	eBiosciences	00-0044-59	
Nunc maxisorp flat bottom 96 well ELISA plates	Nunc/Thermofisher	44-2404-21	high protein binding
Anti-human IgG Ab conjugated to horseradish peroxidase (HRP)	BD Pharmingen / BD Biosciences	55788	
TMB substrate	BD Biosciences	555214	
Streptavidin	Sigma	S0677	
1 mL-HiTrap protein A HP column	GE Healthcare	17-0402-01	
ÄKTA FPLC	GE Healthcare	18190026	
Superdex 200 10/300 GL column	GE Healthcare	17517501	
NGC Quest 10 Plus Chromatography System	BioRad	7880003	