

Materials List for:

Generation of Recombinant Human IgG Monoclonal Antibodies from Immortalized Sorted B Cells

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URL: <https://www.jove.com/video/52830>

DOI: [doi:10.3791/52830](https://doi.org/10.3791/52830)

Materials

Name	Company	Catalog Number	Comments
Histopaque-1077	Sigma-Aldrich	10771	solution containing polysucrose and sodium diatrizoate
FACSAria II cell sorter	BD Biosciences		
96 U-bottom micro well plates	Costar	3799	
Advanced Roswell Park Memorial Institute (RPMI) 1640 medium	Gibco, Life Technologies	12633-020	
30% v/v EBV-containing supernatant of the B95-8 cell line	ATCC	CRL-1612	3.4 x 10 ⁸ copies/ml
CpG2006	Invivogen	ODN 7909	
Wi38 cells	Sigma-Aldrich	90020107	
Interleukin-2	Roche	10799068001	
ELISA plates	Greiner Bio-One, Microlon	655092	
AffiniPure F(ab') ₂ Fragment Goat Anti-Human IgG, Fcγ Fragment Specific (unconjugated)	Jackson ImmunoResearch	109-006-008	
4% non-fat dry milk (Blotting Grade Blocker)	Biorad	170-6404	
Human IgG	Sigma	I 2511	HUMAN IgG purified Immunoglobulin, 5.6 mg/ml
Goat F(ab) ₂ antihuman IgG Fcγ (conjugated with peroxidase (PO))	Jackson ImmunoResearch	109-036-008	
ELISA reader (Perkin Elmer 2030)	Perkin Elmer	2030-0050	
Peroxidase-conjugated AffiniPure Rabbit Anti-Human IgM, Fc _{5μ}	Jackson ImmunoResearch	309-035-095	
SuperScript III Cells Direct cDNA Synthesis System	Invitrogen	18080-200	
Applied Biosystems (ABI) GeneAm PCR System 2700	Applied Biosystems		
High Pure RNA Isolation Kit	Roche	11828665001	
Reverse transcription system kit	Promega	A3500	
Recombinant Taq DNA Polymerase	TAKARA	R001A	
Primers (2 μl)	Sigma		
Ultrapure Agarose	Invitrogen	16500-500	
100 bp ladder	Invitrogen	15628-019	

Quantity One 4.5.2 (Gel Doc 2000)	Biorad	170-8100	
QIAquick PCR purification kit	QIAGEN	28106	
BigDye Terminator v3.1 cycle sequencing kit	Applied Biosystems	4337455	
0.1 ml reaction plate (MicroAMP Optical 96-well)	Applied Biosystems	4346906	
Genetic analyser ABI300	Applied Biosystems	4346906	
DH5 α competent cells (E. coli)	Invitrogen	18263-012	
pFUSEss-CHlg-hG1 (4,493 bp)	Invivogen	pfuse2ss-hchg1	
pFUSEss-CHlg-hG4 (4,484 bp)	Invivogen	pfuse2ss-hchg4	
pFUSE2ss-CLlg-hk (3,875 bp)	Invivogen	pfuse2ss-hclk	
pFUSE2ss-CLlg-hl2 (3,883 bp)	Invivogen	pfuse2ss-hcll2	
SOC medium	Invitrogen	15544-034	
LB-based agar medium supplemented with Zeocin (Fast-Media Zeo Agar)	Invivogen	fas-zn-s	
Terrific Broth (TB)-based liquid medium supplemented with Zeocin (Fast-Media Zeo TB)	Invivogen	fas-zn-l	
DNA Miniprep kit	Omega Bio Technology	D6942-02	
Nanodrop (ND1000 Spectrophotometer)	Nanodrop		
LB-based agar medium supplemented with Blasticidin (Fast-Media Blast Agar)	Invivogen	fas-bl-s	
Terrific Broth (TB)-based liquid medium supplemented with Blasticidin (Fast-Media Blast TB)	Invivogen	fas-bl-l	
EcoRI	New England Biolabs	R0101S	20,000 U/ml, in 10x NEBuffer EcoRI
NheI	New England Biolabs	R0131S	10,000 U/ml, in 10x NEBuffer 2.1
2-Log DNA ladder	New England Biolabs	N3200S	0.1-10.0 kb, 1,000 μ g/ml
XmaI	New England Biolabs	R0180S	10,000 U/ml, in 10x CutSmart Buffer
BsiWI	New England Biolabs	R0553S	10,000 U/ml, in 10x NEBuffer 3.1
AvrII	New England Biolabs	R0174S	5,000 U/ml, in 10x CutSmart Buffer
FastAP Thermosensitive Alkaline Phosphatase	Thermo Scientific	EF0651	1 U/ μ l, in 10x FastAP Buffer
DH5 α competent cells	Invitrogen	18263-012	
PE Mouse Anti-Human IgG	BD Pharmingen	555787	
anti-CD22, PerCP-Cy5.5, Clone: HIB22	Fisher scientific	BDB563942	
QIAprep Spin Miniprep Kit	QIAGEN	27106	
BigDye Terminator v3.1	Applied Biosystems	4337455	