

Materials List for:

Detection and Enrichment of Rare Antigen-specific B Cells for Analysis of Phenotype and Function

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Materials

Name	Company	Catalog Number	Comments
Antigen of interest	variable	variable	At least 100 µg to biotinylate easily; if using protein try to use protein that has been validated by ELISA. It must be carrier protein free.
Biotin for labeling; e.g. EZ link Sulfo-NHS-LC-Biotin	Thermo Scientific	21335	Biotin is available in different formulations, such as those containing various length spacers, so the type used should be determined by the researcher
Streptavidin-Alexa Fluor 647	Invitrogen	S21374	Can obtain from other suppliers.
Anti-Cy5/Anti-Alexa Fluor 647 Microbeads	Miltenyi Biotec	130-091-395	
LS Columns	Miltenyi Biotec	130-042-401	
MACS manual separators	Miltenyi Biotec	variable	
Formaldehyde			Dilute to 2% with PBS; optional if downstream assay requires live cells
PBS without calcium and magnesium			
Ficoll-Paque PLUS	GE Healthcare	17-1440-02	
Whole blood in heparinized collection tubes			
FACS buffer (PBS + 1% BSA + 0.01% sodium azide)			
Separation buffer (PBS + 0.5% BSA + 2 mM EDTA)			
50 ml conical tubes			
15 ml conical tubes			
1.5 ml Eppendorf tubes			
Surface marker reactive antibodies, Fc Block, live/dead discriminating stain, if needed			
ELISPOT supplies, if needed			