

Materials List for:

# Genetically-encoded Molecular Probes to Study G Protein-coupled Receptors

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## Materials

Name	Company	Catalog Number	Comments
Plasmid pSVB. Yam			(Ye <i>et al.</i> , 2008)
Plasmid pcDNA.RS for azF			(Ye <i>et al.</i> , 2009)
Plasmids pMT4.Rho and pcDNA 3.1.CCR5			Optionally contain the amber stop codon (TAG) at a desired position
HEK 293T cells			Adherent cells
Dulbecco's Modified Eagle's Medium (DMEM)	Gibco	10566	
Phosphate Buffered Saline	Gibco	14200	
Fetal Bovine Serum	Gemini Bio-products	100-106	
Lipofectamine Plus	Invitrogen	Lipofectamine: 18324-012 Plus: 11514-015	
<i>p</i> -azido-L-phenylalanine	Chem-Impex International	6162	
			<b>Table 1.</b> Site-specific genetic incorporation of unnatural amino acids into GPCRs materials
Maxima ML-3500S UV-A lamp	Spectronics Corporation		azF is activated by 365-nm light
Hank's Buffered Salt Solution (HBSS)	Gibco	14065	
HEPES	Irvine Scientific	9319	
Bovine serum albumin	Roche	3117405001	
Tritium-labeled ligand			From collaborator (Grunbeck <i>et al.</i> , 2012)
1% (w/v) n-dodecyl- $\beta$ -D-maltoside	Anatrace	D310LA	
1D4-sepharose resin			1D4 mAb immobilized on CNBr-activated sepharose 2B resin
1% (w/v) sodium dodecyl sulfate (SDS)	Fisher Scientific	BP166	
NuPAGE Novex 4 - 12% SDS gels	Invitrogen	NP0322BOX	SDS-PAGE performed on NuPAGE apparatus
Trans-Blot SD apparatus	Biorad		Apparatus for semi-dry transfer
Immobilon polyvinylidene difluoride (PVDF) membrane	Millipore	IPVH00010	
Non-fat powdered milk	Fisher Scientific	NC9934262	
Tween-20	Aldrich	274348	
Ecoscint A	National Diagnostics	LS-273	Scintillation fluid
Scintillation vials	Fisher Scientific	333726	
LKB Wallac 1209 Rackbeta Liquid Scintillation Counter	Perkin Elmer		Beta-Scintillation counter
Anti-rhodopsin 1D4 mAb	National Cell Culture Center	custom	

Horseradish peroxidase (HRP)-conjugated anti-mouse IgG	KPL, Inc.	474-1806	
SuperSignal West Pico Chemiluminescent Substrate	Thermo Scientific	34080	
Hyblot CL AR film	Denville	E3018	
			<b>Table 2.</b> Targeted photocrosslinking materials
0.25% Trypsin	Invitrogen	15050065	
FLAG-triarylphosphine	Sigma	GPHOS1	
M2 FLAG mAb	Sigma	F1804	
anti-CCR5 2D7 mAb	BD Biosciences	555990	
Poly-D-lysine	Sigma	P6407	
96-well plate	Costar	3601	Clear bottom, high binding EIA/RIA
Phosphate Buffered Saline (Calcium, Magnesium)	Gibco	14040	
16% Paraformaldehyde	EMS	28908	
HRP-conjugated	KPL, Inc.	474-1516	
anti-rabbit IgG	KPL, Inc.	474-1516	
Amplex Red	Invitrogen	A12222	
Hydrogen peroxide	DE Healthcare Products	97-93399	
CytoFluor II fluorescence multi-well plate reader	Perseptive Biosystems		
			<b>Table 3.</b> Targeted peptide-epitope tagging and cell surface ELISA materials
Fluorescein-phosphine			(Huber <i>et al.</i> , submitted 2012)
Nonapeptide (C9 peptide)	AnaSpec	62190	Peptide mimicking the 1D4-epitope NH <sub>2</sub> -TETSQVAPA-COOH
1% (w/v) n-dodecyl-β-D-maltoside	Anatrace	D310LA	
1D4-sepharose resin			1D4 mAb immobilized on CNBr-activated sepharose 2B resin
NuPAGE Novex 4 - 12% SDS gels	Invitrogen	NP0322BOX	SDS-PAGE performed on NuPAGE apparatus
Confocal Typhoon 9400 fluorescence scanner	GE Healthcare	discontinued	Scanner with 488-nm wavelength laser
			<b>Table 4.</b> Fluorescent labeling materials