

Materials List for:

Optimizing the Genetic Incorporation of Chemical Probes into GPCRs for Photo-crosslinking Mapping and Bioorthogonal Chemistry in Live Mammalian Cells

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Materials

Name	Company	Catalog Number	Comments
Chemicals			
Acryamide/Bisacrylamide 30% (37,5:1)	Carl Roth	3029.1	
Ammonium persulfate (APS)	Carl Roth	9592.2	
p-Azidophenylalanine (Azi)	Bachem	F-3075.0001	
Boric acid	Sigma Aldrich	B6768	
Bromphenolblue	Sigma-Aldrich	B0126-25G	
Bovine serum albumine (BSA)	Carl Roth	8076.2	
Carbobenzyloxy-L-lysine (Lys(Z))	NovaBiochem	8540430100	
Cyclooctyne-L-lysine (SCOK)	Sichem	SC-8000	
DMEM	Life Technologies	41966052	
DMSO	Carl Roth	A994.2	
DTT	Carl Roth	6908.1	
enhanced chemiluminescence reagent (ECL)	home-made		10 mg/l luminol in 0.1 M Tris-HCl pH 8.6 ; 1100 mg/l p-coumaric acid in DMSO ; 30 % H ₂ O ₂ (1,000 : 100 : 0.3)
EDTA	Carl Roth	8043.1	
EGTA	Carl Roth	3054.1	
endo-bicyclo[6.1.0]nonyne-L-lysine (BCNK)	Sichem	SC-8014	
FBS	Thermo Fisher (Gibco)	10270106	
FluoroBrite DMEM	Thermo Fisher (Gibco)	A1896701	
Glycerol	Carl Roth	7533.1	
Glycin	Carl Roth	3908.3	
HEPES	Carl Roth	9105.3	
Hoechst 33342	Sigma Aldrich	B2261	
KCl	Carl Roth	6781.3	
Lipofectamine 2000	Thermo Fisher	11668019	
Luminol	Appllichem	A2185,0005	
Methanol	Carl Roth	0082.3	
MgCl ₂	Carl Roth	2189.2	
NaCl	Carl Roth	HN00.2	

Na-Lactate	Sigma-Aldrich	71718-10G	
NaOH	Grüssing	121551000	
PBS	Sigma-Aldrich	P5493-1L	
p-Coumaric acid	Sigma-Aldrich	C9008-1G	
poly-D-lysine hydrobromide	Corning	354210	
PEI	Polysciences	23966	
Penicillin/Streptomycin	Thermo Fisher (Gibco)	11548876 (15140-122)	
PMSF	Carl Roth	6367.1	
PNGase F	NEB	P0704L	
Protease Inhibitor	Roche	11873580001	
PVDF membrane Immobilon-P	Millipore	IPVH00010	
Skim Milk Powder	Sigma	70166	
Sodium dodecyl sulfate (SDS)	Carl Roth	CN30.2	
Tetrazine-Cy3	Jena Bioscience	CLK-014-05	
Tetramethylethylenediamine (TEMED)	Carl Roth	2367.3	
trans-Cyclooctene-L-lysine (TCO*K)	Sichem	SC-8008	
TRIS	Sigma-Aldrich	T1503	
Triton X-100	Carl Roth	3051.4	
Trypsin 2.5%	Thermo Fisher (Gibco)	15090046	
Tween 20	Carl Roth	9127.2	
Wasserstoffperoxid (30%)	Merck	1.07210.0250	
Cell lines			
HEK293 cells	German Collection of Microorganisms and Cell Cultures GmbH (DSMZ)	ACC-305	
HEK293T cells	German Collection of Microorganisms and Cell Cultures GmbH (DSMZ)	ACC-635	
Equipment			
Crosslinker Bio-Link 365 nm	Bio-Budget Technologies GmbH	40-BLX-E365	5 x 8 Watt tubes
Plate Reader BMG LABTECH FLUOstar Omega	BMG LABTECH		
Plasmids			
Plasmid E2AziRS	The humanized gene for E2AziRS was synthesized by Genart (Life Technologies)		Plasmid containing 4 tandem copies of the suppressor tRNA Bst-Yam driven by the human U6 promoter and one copy of a humanized gene for the enhanced variant of the Azi-tRNA synthetase (EAziRS) driven by a PGK promoter
POI-TAG mutant plasmids			Plasmid encoding the POI driven by the CMV promoter, C-terminally fused to the FLAG-tag, bearing a TAG codon at the desired position
CRF1R-95TAG-EGFP			Cloned in the MCS of pcDNA3.1
HA-PTH1R-79TAG-CFP			Cloned in the MCS of pcDNA3.1
Arrestin3-FLAG	Synthesized by Genart (Life Technologies)		Cloned in the MCS of pcDNA3.1
Antibodies			

Anti-FLAG-HRP M2 antibody conjugate	Sigma-Aldrich	A8592	monoclonal, produced in mouse clone M2
Goat-anti-rabbit-HRP antibody	Santa Cruz	sc-2004	
Rabbit-anti-CRF antibody	home-made	PBL #rC69	polyclonal Turnbull, A.V., Vaughan, J., Rivier, J.E., and Vale, W.W. Endocrinology, 140, (1), 71-78 (1999)
Rabbit-anti-Ucn1 antibody	home-made	PBL #5779	polyclonal Turnbull, A.V., Vaughan, J., Rivier, J.E., and Vale, W.W. Endocrinology, 140, (1), 71-78 (1999)