

Materials List for:

Efficient and Site-specific Antibody Labeling by Strain-promoted Azide-alkyne Cycloaddition

Sanggil Kim¹, Wooseok Ko¹, Hyunji Park¹, Hyun Soo Lee¹

¹Department of Chemistry, Sogang University

Correspondence to: Hyun Soo Lee at hslee1976@gmail.com

URL: <https://www.jove.com/video/54922>

DOI: [doi:10.3791/54922](https://doi.org/10.3791/54922)

Materials

Name	Company	Catalog Number	Comments
1. Plasmid Construction			
plasmid pBAD_HerFab_L177TAG			Optionally contain the amber stop codon (TAG) at a desired position. Ko, W. <i>et al.</i> Efficient and Site-Specific Antibody Labeling by Strain-promoted Azide-Alkyne Cycloaddition. <i>BKCS</i> . 36 (9) , 2352-2354, doi: 10.1002/bkcs.10423, (2015)
plasmid pEvol-AFRS			Young, T. S., Ahmad, I., Yin, J. A., and Schultz, P. G. An enhanced system for unnatural amino acid mutagenesis in <i>E. coli</i> . <i>J. Mol. Biol.</i> 395 (2) , 361-374, doi: 10.1016/j.jmb.2009.10.030, (2010)
DH10B	Invitrogen	C6400-03	Expression Host
Plasmid Mini-prep kit	Nucleogen	5112	200/pack
Agarose	Intron biotechnology	32034	500 g
Ethidium bromide	Alfa Aesar	L07482	1 g
LB Broth	BD Difco	244620	500 g
2. Culture Preparation			
2.1 Electroporation			
Micro pulser	BIO-RAD	165-2100	
Micro pulser cuvette	BIO-RAD	165-2089	0.1 cm electrode gap, pkg. of 50
Ampicillin Sodium	Wako	018-10372	25 g
Chloramphenicol	Alfa Aesar	B20841	25 g
Agar	SAMCHUN	214230	500 g
SOC medium	Sigma	S1797	100 mL
3. Expression and Purification of HerFab-L177AF			
3.1 Expression of Herfab-L177AF			
p-azido-L-phenylalanine (AF)	Bachem	F-3075.0001	1 g
L(+)-Arabinose, 99%	Acros	104981000	100 g
Hydrochloric acid, 35~37%	SAMCHUN	H0256	500 mL
3.2 Cell Lysis			
Tris(hydroxymethyl)aminomethane, 99%	SAMCHUN	T1351	500 g
EDTA disodium salt dihydrate, 99.5%	SAMCHUN	E0064	1 kg
Sucrose	Sigma	S9378	500 g

Lysozyme	Siyaku	126-0671	1 g
3.3 Ni-NTA Affinity Chromatography			
Ni-NTA resin	QIAGEN	30210	25 mL
Polypropylene column	QIAGEN	34924	50/pack, 1 mL capacity
Imidazole, 99%	SAMCHUN	I0578	1 kg
Sodium phosphate monobasic, 98%	SAMCHUN	S0919	1 kg
Sodium Chloride, 99%	SAMCHUN	S2907	1 kg
4. Conjugation of Purified HerFab-L177AF with Alkyne Probes Using Strain-promoted Azide-alkyne Cycloaddition (SPAAC)			
Cy5.5-ADIBO	FutureChem	FC-6119	1 mg
5. Purification of Labeled HerFab			
Amicon Ultra 0.5 mL Centrifugal Filters	MILLIPORE	UFC500396	96/pack, 500 µL capacity
6. SDS-PAGE Analysis of Labeled HerFab and Fluorescent Gel Scanning			
1,4-Dithio-DL-threitol, DTT, 99.5%	Sigma	10708984001	10 g
NuPAGE LDS Sample Buffer, 4x	Thermofisher	NP0007	10 mL
MES running buffer	Thermofisher	NP0002	500 mL
Nupage Novex 4-12% SDS PAGE gels	Thermofisher	NO0321	12-well
Coomassie Brilliant Blue R-250	Wako	031-17922	25 g
Typhoon 9210 variable mode imager	Amersham Biosciences		