

Measuring Nucleotide Binding to Intact, Functional Membrane Proteins in Real Time

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Citation

Usher, S.G., Ashcroft, F.M., Puljung, M.C. Measuring Nucleotide Binding to Intact, Functional Membrane Proteins in Real Time. *J. Vis. Exp.* (), e61401, doi:10.3791/61401 (2021).

Date Published

March 11, 2021

DOI

10.3791/61401

URL

jove.com/video/61401

Materials

Name	Company	Catalog Number	Comments
T75 tissue-culture treated flask	StarLab	CC7682-4875	
0.1% w/v poly-L-lysine	Sigma-Aldrich	P8920	
30 mm borosilicate cover glass slips	VWR	631-0174	
35 mm non-treated sterile dishes	CytoOne	CC7672-3340	
35 mm cover glass bottom dish	WPI	FD35-PDL-100	
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	31966021	
Foetal bovine serum (FBS)	Gibco	10500-064	
Penicillin/Streptomycin	Gibco	15140-122	
TrypLE select (trypsin)	Gibco	12563-011	Trypsin/EDTA reagent
Phosphate buffered saline (PBS)	Gibco	14040-091	
UltraPure distilled water	Invitrogen	10977-035	
HEK293T cells	ATTC	CRL-3216	Used between passages 5-30
ANAP-TFA	AsisChem	ASIS-0014	Reconstituted in 30 mM NaOH to a final concentration of 1 mM
pANAP expression plasmid	Addgene	Plasmid #48696	Encodes tRNA/tRNA synthetase pair for expression of ANAP-tagged protein
peRF1-E55D	Chin Lab (MRC Laboratory of Molecular Biology, Cambridge, UK)	Jason Chin: DOI: 10.1021/ja5069728	Encodes dominant-negative eukaryotic ribosomal release factor
TransIT-LT1	Mirus Bio	MIR 2300	Lipopolyplex transfection reagent
Thick-walled borosilicate glass capillaries	Harvard Apparatus	GC150F-15	
Tetramethylrhodamine-5-maleimide	Sigma-Aldrich	94506	
TNP-ATP	Jena Bioscience	NU-221L	Delivered at 10 mM in water
Nikon Eclipse TE2000-U inverted microscope	Nikon		
60x water immersion objective (1.4 NA)	Nikon	MRD07602	
4-Wavelength High-Power LED Head	ThorLabs	LED4D245	385/490/565/625 nm LEDs

Four-Channel LED Driver	ThorLabs	DC4100	
390/18 nm band-pass excitation filter	ThorLabs	MF390-18	For ANAP excitation
400 nm long-pass emission filter	ThorLabs	FEL0400	For imaging ANAP spectra
416 nm edge dichroic	ThorLabs	MD416	For imaging ANAP spectra
460/60 nm band-pass emission filter	ThorLabs	MF460-60	Suggested wide band-pass filter for imaging ANAP fluorescence (Figure 4B)
470/10 nm band-pass emission filter	ThorLabs	FB470-10	Suggested narrow band-pass filter for imaging ANAP fluorescence (Figure 4B)
531/40 band-pass excitation filter	Brightline	FF01-531/40-25	For orange fluorescent protein (OFP) excitation
540/25 nm band-pass excitation filter	Chroma	D540/25X	For tetramethylrhodamine-5-maleimide (TMRM) excitation
562 nm edge dichroic	Semrock	FF562-Di03	For imaging OFP fluorescence
565 nm edge dichroic	Chroma	565DC	For imaging TMRM fluorescence
593/40 nm band-pass excitation filter	Brightline	FF01-387/11-25	For imaging OFP fluorescence
605/55 nm band-pass emission filter	Chroma	D605/55M	For imaging TMRM fluorescence
IsoPlane-160 Imaging Spectrometer	Princeton Instruments	IsoPlane-160	
PIXIS 400BR_eXcelon Camera	Princeton Instruments	PIXIS: 400BR_eXcelon	
Axopatch 200B amplifier	Molecular Devices	Axopatch 200B-2	
Digidata 1440A digitizer	Molecular Devices	Digidata 1440A	
Probe sonicator	Sonics & Materials	VC-50	For unroofing
REGLO digital peristaltic pump	Ismatec	ISM 832	For bath perfusion
Microvolume perfusion system	ALA Scientific Instruments	ALA μ Flow-8	For TNP-ATP perfusion
pClamp 10.6.2	Molecular Devices		Recording and analysing currents
Lightfield 5.20.1507	Princeton Instruments		Acquisition software for images and spectra
Matlab	Mathworks		For data analysis
Python 3.8.1	Python Software Foundation		For data analysis