

An Optimized Single-Molecule Pull-Down Assay for Quantification of Protein Phosphorylation

Elizabeth M. Bailey¹, Emanuel Salazar-Cavazos¹, Rachel M. Grattan¹, Michael J. Wester², David J. Schodt², Julian A. Rojo¹, Keith A. Lidke^{2,3}, Diane S. Lidke^{1,3}

¹Department of Pathology, University of New Mexico School of Medicine ²Department of Physics and Astronomy, University of New Mexico

³Comprehensive Cancer Center, University of New Mexico Health Sciences Center

Corresponding Author

Diane S. Lidke

dldike@salud.unm.edu

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Materials

Name	Company	Catalog Number	Comments
1.5 mL microcentrifuge tubes	MTC Bio	C2000	
10 mM Tris-HCl pH 7.4			
10 mM Tris-HCl pH 8.0/ 50 mM NaCl			T50 Buffer
100 mm Tissue Culture dish	CELLTREAT	229620	Storage of piranha etched glass/ arrays
15 mL conical tube			
16% Paraformaldehyde Aqueous Solution	Electron Microscopy Sciences	15710	Hazardous
50 mL conical tube			Functionalized Glass storage/ KOH reuse
50 mM Tris-HCl pH 7.2/150 mM NaCl			Lysis Buffer Component
60 mm Tissue Culture dish	Corning	430166	
8% Glutaraldehyde Aqueous Solution	Electron Microscopy Sciences	16020	Hazardous
Acetone (C ₃ H ₆ O)	Millipore Sigma	270725	Hazardous
Alexa Fluor 647 NHS Ester	Thermo Fisher Scientific	A-20006	
Animal-Free Recombinant Human EGF	Peptrotech	AF-100-15	
Anti-Human EGFR (External Domain) – Biotin	Leinco Technologies, Inc	E101	
Anti-p-Tyr Antibody (PY99) Alexa Fluor 647	Santa Cruz Biotechnology	sc-7020 AF647	
Bath-sonicator	Branson	1200	
BCA Protein Assay Kit	Pierce	23227	
Biotin-PEG	Laysan Bio	Biotin-PEG-SVA, MW 5,000	
Bovine serum albumin	Gold Biotechnology	A-420-1	Tyrode's Buffer Component
Buchner funnel			
Bunsen burner			

Calcium Chloride (CaCl ₂)	Millipore Sigma	C4901	Tyrode's Buffer Component
Cell Scraper	Bioworld	30900017-1	
Conical Filtering Flask	Fisher Scientific	S15464	
Coplin Jar	WHEATON	900470	
Countess II Automated Cell Counter	Thermo Fisher Scientific	AMQAX1000	
Coverslips 24 x 60 #1.5	Electron Microscopy Sciences	63793	
DipImage			https://diplib.org/
DMEM	Caisson Labs	DML19-500	
emCCD camera	Andor iXon		
Fetal Bovine Serum, Optima	Bio-Techne	S12450H	Heat Inactivated
Fusion 360 software	Autodesk		
Geneticin G418 Disulfate	Caisson Labs	G030-5GM	
Glacial Acetic Acid (CH ₃ COOH)	JT Baker	JTB-9526-01	Hazardous
Glass serological pipettes			
Glass Stir Rod			
Glucose (D-(+)-Glucose)	Millipore Sigma	D9434	Tyrode's Buffer Component
Halt Phosphatase and Protease Inhibitor Cocktail (100X)	Thermo Fisher Scientific	78446	Lysis Buffer Component
HEPES	Millipore Sigma	H3375	Tyrode's Buffer Component
Hydrochloric Acid (HCl)	VWR	BDH7204-1	Hazardous
Hydrogen Peroxide (H ₂ O ₂) (3%)		HX0645	
Hydrogen Peroxide (H ₂ O ₂) (30%)	EMD Millipore	HX0635-2	
Ice			
IGEPAL CA-630 (NP-40)	Sigma Aldrich	I8896	Lysis Buffer Component
ImmEdge Hydrophobic Barrier Pen	Vector Laboratories	H-4000	
Immersol 518F immersion oil	Zeiss	444960-0000-000	
in-house vacuum line			
L-glutamine	Thermo Fisher Scientific	25030-164	
Magnesium Chloride Hexahydrate (MgCl ₂ ·6H ₂ O)	MPBio	2191421	Tyrode's Buffer Component
Matlab	Mathworks		Curve Fitting Toolbox, Parallel Computing Toolbox, and Statistics and Machine Learning toolbox
Methanol (CH ₃ OH)	IBIS Scientific	MX0486-1	Hazardous
Milli-Q water			
Mix-n-Stain CF Dye Antibody Labeling Kits	Biotium	92245	Suggested conjugation kit
mPEG	Laysan Bio	mPEG-succinimidyl valerate, MW 5,000	
N-(2-aminoethyl)-3-aminopropyltrimethoxysilane	UCT United Chemical	A0700	Hazardous
Nanogrid	Miraloma Tech		
NeutrAvidin Biotin Binding Protein	Thermo Fisher Scientific	31000	
Nitrogen (compressed gas)			
NVIDIA GPU with CUDA			Look for compatibility at https://www.mathworks.com/help/parallel-computing/gpu-support-by-release.html
Olympus iX71 Microscope	Olympus		
Parafilm M Sealing Film	The Lab Depot	HS234526C	

PBS pH 7.4	Caisson Labs	PBL06	
PC-200 Analog Hot Plate	Corning	6795-200	
Penicillin-Streptomycin (10,000 U/ mL)	Thermo Fisher Scientific	15140-163	
Phospho-EGF Receptor (Tyr1068) (1H12) Mouse mAb	Cell Signaling Technology	2236BF	
Potassium Chloride (KCl)	Millipore Sigma	529552	Tyrode's Buffer Component
Potassium Hydroxide (KOH)	Millipore Sigma	1050330500	Hazardous
Premium PLA Filament, 1.75 mm diameter	Raise 3D	PMS:2035U/RAL:3028	Printing temperature range: 205-235 °C
Pro2 3D printer	Raise 3D		
Pyrex 1 L beaker			
PYREX 100 mL storage bottles	Corning	1395-100	CH ₃ OH/C ₃ H ₆ O reuse
Pyrex 250 mL beakers			
Pyrex 4 L beaker			
Quad-view Image Splitter	Photometrics	Model QV2	
Refrigerated centrifuge	Eppendorf	EP-5415R	
RevCount Cell Counters, EVE Cell Counting Slides	VWR	10027-446	
Semrock emission filters: blue (445/45 nm), green (525/45 nm), red (600/37 nm), far-red (685/40 nm)	Semrock	LF405/488/561/635-4X4M-B-000	
Serological pipette controller			
Serological Pipettes			
smite single molecule analysis package			https://github.com/LidkeLab/smite.git
Sodium Bicarbonate (NaHCO ₃)	Sigma Aldrich	S6014	Hazardous
Sodium Borohydride (NaBH ₄)	Millipore Sigma	452874	Tyrode's Buffer Component
Sodium Chloride (NaCl)	Millipore Sigma	S9625	Activate by successive heat and pH cycling
Sodium Hydroxide	VWR	BDH3247-1	
Sodium Orthovanadate (Na ₃ VO ₄)	Millipore Sigma	S6508	Hazardous
Sulfuric Acid (H ₂ SO ₄)	Millipore Sigma	258105	Hazardous
TetraSpeck Microspheres	Thermo Fisher Scientific	T7279	multi-fluorescent beads
Tris (Trizma) base	Millipore Sigma	T1503	
Trypan blue stain, 0.4%	Thermo Fisher Scientific	15250061	
Trypsin-EDTA 0.05%	Thermo Fisher Scientific	25300120	