

Fluorescence-coupled Micropipette Aspiration Assay to Investigate Red Blood Cell Mechanosensing

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Materials

Name	Company	Catalog Number	Comments
µManager	Micro-Manager		Version 2.0.0
1 mL Syringe	Terumo	210320D	Cooperate with the Microfil
200 µL Pipette	Eppendorf	3123000055	Red blood cell preparation
22 x 40 mm Cover Slips	Knittel Glass	MS0014	Cell chamber assembly
50 mL Syringe	Terumo	220617E	Connect to the water tower
Calcium Chloride (CaCl ₂)	Sigma-Aldrich	C1016	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
Centrifuge 5425	Eppendorf	5405000280	Red blood cell preparation
Clexane	Sigma-Aldrich	1235820	To prevent clotting of the collected blood. 10,000 U/mL
DAQami	Diligent		
Fluorescence light source	CoolLED	pE-300	Micropipette aspiration hardware system
Glass capillary	Narishige	G-1	Micropipette manufacture
Glucose	Sigma-Aldrich	G8270	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
Hepes	Thermo Fisher	15630080	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
High speed GigE camera	Manta	G-040B	Micropipette aspiration hardware system
High speed pressure clamp	Scientific Instrument	HSPC-2-SB	Cooperate with the pressure pump

High speed pressure clamp head stage	Scientific Instrument	HSPC-2-SB	Cooperate with the pressure pump
Imaris	Oxford Instruments		
Inverted Microscopy	Olympus	Olympus IX83	Micropipette aspiration hardware system
Microfil	World Precision Instruments	MF34G-5	34 G (67 mm Long) Remove air bubble in the cut micropipette and test the opening of the pipette tip
Micropipette Puller	Sutter instrument	P1000	Micropipette manufacture
Milli Q EQ 7000 Ultrapure Water Purification System	Merck Millipore	ZEQ7000T0C	Carbonate/bicarbonate buffer & Tryode's buffer preparation
Pipette microforge	Narishige	MF-900	Micropipette manufacture
Potassium Chloride (KCl)	Sigma-Aldrich	P9541	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
Pressure Pump	Scientific Instrument	PV-PUMP	Induce controlled pressure during experiment
Prime 95B Camera	Photometrics	Prime 95B sCMOS	Fluorescent imaging
Rotary wheel remote unit	Sensapex	uM-RM3	Control panel for micropipette position adjustment
Scepter 3.0 Handheld Cell Counter	Merck Millipore	PHCC340KIT	Automatic cell counter
Sodium Bicarbonate (NaHCO ₃)	Sigma-Aldrich	S5761	Carbonate/bicarbonate buffer preparation - 2.65 g of NaHCO ₃ with 2.1 g of Na ₂ CO ₃ in 250 mL of Mili Q water - Final pH = 8-9.
Sodium Carbonate (Na ₂ CO ₃)	Sigma-Aldrich	S2127	Carbonate/bicarbonate buffer preparation - 2.65 g of NaHCO ₃ with 2.1 g of Na ₂ CO ₃ in 250 mL of Mili Q water - Final pH = 8-9.
Sodium Chloride (NaCl)	Sigma-Aldrich	S7653	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
Sodium Phosphate Monobasic Monohydrate (NaH ₂ PO ₄ • H ₂ O)	Sigma-Aldrich	S9638	Tryode's buffer preparation - 12 mM NaHCO ₃ , 10 mM HEPES, 0.137 M NaCl, 2.7 mM KCl, and 5.5 mM D-glucose supplemented with 1 mM CaCl ₂ . Final pH = 7.2
Touch screen control unit	Sensapex	uM-TSC	Control panel for micropipette position adjustment
X dry Objective	Olympus	Olympus 60x/0.70 LUCPlanFL	Micropipette aspiration hardware system