

Materials List for:

# Analysis of Protein-protein Interactions and Co-localization Between Components of Gap, Tight, and Adherens Junctions in Murine Mammary Glands

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## Materials

Name	Company	Catalog Number	Comments
Mice strain and stage	St. Constant, Quebec, Canada		C57BL/6 Femals; pregnancy day 18 (P18) and lactation day 14 (L14), Charles River Canada
PBS 10x (stock)			1) Dissolve 80 g NaCl (F.W.: 58.44), 2 g KCl (F.W. 74.55), 26.8 g Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O (F.W. 268.07) and 2.4 g KH <sub>2</sub> PO <sub>4</sub> (F.W.:136.09) in 800 mL distilled water; 2) Adjust the PH to 7.4; 3) Add water to reach to the 1 L final volume.
TBS 10x (stock)			1) Dissolve 60.5 g TRIS, 87.6 g NaCl in 800 mL distilled water; 2) Adjust the pH to 7.5; 3) Add water to reach to the 1 L final volume.
<b>Part 1: Immunofluorescence</b>			
Freezing media	VWR International, Ville Mont-Royal, QC, Canada	95067-840	VMR frozen sections compound
Microtome	Mississauga, ON, Canada	956640	Microm HM525, Thermo fisher scientific HM525 NX Cryostat 115 V 60 Hz
Blades	C.L. Sturkey, Inc. Les Produits Scientifiques ESBE St-Laurent, QC, Canada	BLM1001C	High profile gold coated blades
Pap pen	Cedarlane, Burlington, ON, Canada	8899	Super PAP Pen, Thermo fisher scientific
Microscopic slides	Fisher Scientific, Burlington, ON, Canada	12-550-15	Fisherbrand Superfrost Plus Microscope Slides
Formaldehyde	BioShop Canada Inc, Burlington, ON, Canada	FOR201.1	Forlmadehyde
Bovine Serum Albumin (BSA)	Santa Cruz Biotechnology, Inc, California, USA		
Blocking solution			3% BSA in TBS
Wash solution			TBS-Tween 20 0.1%
Polysorbate 20	Oakville, ON, Canada	P 9416	Tween 20, Sigma-Aldrich
Mounting media	Cedarlane, Burlington, ON	17984-25(EM)	Fluoromount-G
First & secondary antibodies	Cell Signaling, Beverly, MA, USA	See Comments	E-Cadherin (4A2) Mouse mAb (#14472s) 1/50 (Cell Signaling)

			with anti-mouse IgG Fab2 Alexa Fluor 555 (#4409s), Cell Signaling
First & secondary antibodies	Life technologies, Waltham, MA, USA & Cell Signaling, Beverly, MA, USA	See Comments	Claudin-7 (#34-9100) 1/100 (Life Technologies) with anti-rabbit IgG Fab2 Alexa Fluor 488 (#4412s) (Cell Signaling)
First & secondary antibodies	Santa Cruz Biotechnology, Inc, California, USA; Fischer Scientific, Burlington, ON, Canada	See Comments	$\beta$ -Catenin Antibody (C-18): sc-1496 (SANTA CRUZ) with anti-Goat IgG (H+L) Alexa Fluor 568 (#A11057), Molecular Probe (Fisher Scientific)
First & secondary antibodies	Life technologies, Waltham, MA, USA & Cell Signaling, Beverly, MA, USA	See Comments	Connexin26 (#33-5800) 1/75 (Life Technologies) with anti-mouse IgG Fab2 Alexa Fluor 647 (#4410s)
Nuclei stain	Fisher Scientific, Burlington, ON, Canada	D1306	DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) 1/1,000 in PBS
Fluorescent microscope	Nikon Canada, Mississauga, On, Canada		Nikon A1R+ confocal microscopic laser equipped with a spectral detector
Software of IF images analysis	Nikon Canada, Mississauga, On, Canada		NIS-elements software (version 4)
<b>Part 2: Immunoprecipitation</b>			
Triple-detergent Lysis buffer (100 mL) pH=8.0			1) Mix 50 mM TRIS (F.W.: 121.14), 150 mM NaCl (F.W.: 58.44), 0.02% Sodium Azide, 0.1% SDS, 1% NONIdET P40, 0.5% Sodium Deoxycholate in 80 mL distilled H <sub>2</sub> O. 2) Adjust the pH to 8.0 with HCl 6 N (~0.5 mL). 3) Adjust the volume to 100 mL. Keep it in fridge. At the day of protein extraction, use 1/100 NaVO <sub>3</sub> , 1/100 protease/phosphatase inhibitor and 1/25 NAF in calculated amount of Triple detergent lysis buffer: Sodium Fluoride (stock) solution 1.25 M (F.W.: 41.98), Sodium Orthovanadate (stock) Solution 1 M (F.W.: 183.9)
Protease/phosphatase inhibitor	Fisher Scientific, Burlington, ON	78441	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100x)
Protein dosage	Thermo Scientific, Rockford, Illinois, USA	23225	Pierce BCA protein assay kit
Tissue grinder	Fisher Scientific, Burlington, ON	FTH-115	Power 125, Model FTH-115
Magnetic beads and stand	Millipore, Etobicoke, ON, Canada		PureProteome Protein G Magnetic Bead System (LSKMAGG02)
Wash solution for IP			PBS or PBS-Tween20 0.1% depending to the step
Primary antibodies for immunoprecipitation	Cell Signaling, Beverly, MA, USA	See Comments	IgG Rabbit (rabbit (DA1E) mAb IgG Isotype control (#3900s) (Cell Signaling) 0.5 $\mu$ L/200 $\mu$ L
Primary antibodies for immunoprecipitation	Cell Signaling, Beverly, MA, USA	See Comments	IgG Mouse mouse (G3A1) mAb IgG Isotype control (#5415s) (Cell Signaling) 0.5 $\mu$ L/200 $\mu$ L
Primary antibodies for immunoprecipitation	Sigma-Aldrich, Oakville, ON, Canada	See Comments	Connexin43 (#C6219) (Sigma-Aldrich) 4 $\mu$ L/200 $\mu$ L

Primary antibodies for immunoprecipitation	Cell Signaling, Beverly, MA, USA	See Comments	E-cadherin (4A2) Mouse mAb (#14472s) (Cell Signaling) 1 $\mu$ L/200 $\mu$ L
Laemmli buffer	BIO-RAD, Mississauga, Ontario, Canada	1610747	4x Laemmli Sample Buffer (Add $\beta$ -mercaptoethanol following manufacturer recommendation)
Acidic glycine	Fisher Scientific, Burlington, ON	PB381-5	0.2 M glycine; adjust pH=2.5 with HCl
Tris	Fisher Scientific, Burlington, ON	BP152-1	1 M (pH=8)
SDS-PAGE acrylamide gels	BIO-RAD, Mississauga, ON, Canada	1610180 -5	TGX Stain-Free FastCast Acrylamide Solutions (7.8%, 10%, 12%)
Running buffer 10x	BIO-RAD, Mississauga, ON, Canada	1704272	Tris 30.3 g/glycine 144.1 g /SDS 10 g in 1 L distilled water
Membranes	BIO-RAD, Mississauga, ON, Canada	1704272	PVDF membranes, Trans-Blot Turbo RTA Mini PVDF Transfer Kit
Transfer method	BIO-RAD, Mississauga, ON, Canada	1704155	Trans-Blot Turbo Transfer System
Dry Milk	Smucker Food of Canada Co, Markham, ON, Canada		Fat Free Instant Skim Milk Powder, Carnation
Blocking solution for blots			5% dry milk in TBS-Tween 20 0.1%
Washing solutions for blots			TBS-Tween 20 0.1%
Primary and secondary antibodies for blots (10 mL)	Sigma-Aldrich, Oakville, Ontario & Abcam, Toronto, ON, Canada	See Comments	Connexin43 (#C6219) (Sigma-Aldrich) 1/2,500 with HRP-conjugated Veriblot for IP secondary antibody (ab131366) 1/5,000 (Abcam, Toronto, ON, Canada)
Primary and secondary antibodies for blots (10 mL)	Cell Signaling, Beverly, MA, USA & Abcam, Toronto, ON, Canada	See Comments	E-cadherin (24E10) rabbit mAb 1/1,000 (#3195s) (Cell Signaling) 1/1,000 with HRP-conjugated Veriblot for IP secondary antibody (ab131366) 1/5,000 (Abcam, Toronto, ON, Canada)
Primary and secondary antibodies for blots (10 mL)	Life technologies, Waltham, MA, USA & Abcam, Toronto, ON, Canada	See Comments	Claudin-7 (#34-9100) (Life technologies) 1/1,000 with HRP-conjugated Veriblot for IP secondary antibody (ab131366) 1/5,000 (Abcam, Toronto, ON, Canada)
Primary and secondary antibodies for blots (10 mL)	Life technologies, Waltham, MA, USA & Abcam, Toronto, ON, Canada	See Comments	Claudin3 (#34-1700) (Life technologies) 1/1,000 with HRP-conjugated Veriblot for IP secondary antibody (ab131366) 1/5,000 (Abcam, Toronto, ON, Canada)
Luminol solution for signal detection on blots	BIO-RAD, Mississauga, ON, Canada	1705061	Clarity Western ECL Blotting Substrate
Imaging blots	BIO-RAD, Mississauga, ON, Canada	1708280	ChemiDoc MP imaging system
Analyzing blots	BIO-RAD, Mississauga, ON, Canada		ImageLab 5.2 software