

Materials List for:

Detection of *In Situ* Protein-protein Complexes at the *Drosophila* Larval Neuromuscular Junction Using Proximity Ligation Assay

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Materials

Name	Company	Catalog Number	Comments
Forceps (fine #5)	Almedic	A10-704	
Sylgard Disc	World Precision Instruments	SYLG184	Mix elastomer base and curing agent in a 10:1 ratio. Set for 30 min. Pour into a mold (e.g. use a 12-well cell culture plate). Let cure for at least 24 hr. Adhere to the lid of a 60 x 15 mm Petri dish lid when dissecting.
Minutien Pins (0.0125 mm tip diameter)	Fine Science Tools	26002-10	
Microdissection Scissors (ultra fine)	Fine Science Tools	15200-00	
Platform Slides			Glue two 22 x 22 mm ² coverslips onto a microscope slide with clear nail polish, leaving a <20 mm gap in between for sample mounting.
w ¹¹¹⁸	Bloomington Drosophila Stock Center	3605	
hts ⁰¹¹⁰³	Bloomington Drosophila Stock Center	10989	Stock was re-balanced over a GFP balancer so that homozygous mutants can be selected based on the absence of GFP signal.
1x PBS (Phosphate Buffered Saline): 3 mM NaH ₂ PO ₄ , 7 mM Na ₂ HPO ₄ , 130 mM NaCl, pH 7.0			NaH ₂ PO ₄ (Caledon Laboratories - 8180-1), Na ₂ HPO ₄ (Caledon - 8120-1), NaCl (Caledon - 7560-1)
Bouin's Solution	Sigma-Aldrich	HT10132	
4% PFA (Paraformaldehyde): 4% PFA in 1x PBS			PFA (Anachemia Science - 66194-300). See doi:10.1101/pdb.rec9959 Cold Spring Harb Protoc 2006 for instructions on how to make the solution.

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1x PBT (Phosphate Buffered Saline with Triton): 1x PBS with 0.01% Triton			Triton X-100 (Sigma-Aldrich - T8787)
1% BSA (Bovine Serum Albumin): 1% BSA in 1x PBT			BSA (Bioshop Canada - ALB001). Store at 4 °C.
mouse anti-Dlg (Discs large)	Developmental Studies Hybridoma Bank	4F3	Use at a 1:10 dilution in 1% BSA.
rabbit anti-HtsM (Hu-li tai shao)			Provided by Dr. Lynn Cooley (Yale University). Use at a 1:250 dilution in 1% BSA.

rabbit anti-Pak (p21-activated kinase)			Provided by Dr. Nicholas Harden (Simon Fraser University). Use at a 1:500 dilution in 1% BSA.
mouse anti-Wg (Wingless)	Developmental Studies Hybridoma Bank	4D4	Use at a 1:5 dilution in 1% BSA.
goat anti-Hrp (Horseradish peroxidase)	Jackson ImmunoResearch	123-065-021	Use at a 1:200 dilution in 1% BSA.
FITC-conjugated donkey anti-goat	Jackson ImmunoResearch	705-095-003	Use at a 1:200 dilution in 1% BSA.
Duolink In Situ PLA Probe anti-mouse MINUS	Sigma-Aldrich	DUO92004	
Duolink In Situ PLA Probe anti-rabbit PLUS	Sigma-Aldrich	DUO92002	
Duolink In Situ Detection Reagents Red	Sigma-Aldrich	DUO92008	
1x Wash Buffer A: 0.01 M Tris, 0.15 M NaCl, 0.05% Tween 20, pH 7.4	Sigma-Aldrich	DUO82049	Tris (Caledon Laboratories - 8980-1), NaCl (Caledon - 7560-1), Tween 20 (Fisher Scientific - BP337)
1x Wash Buffer B: 0.2 M Tris, 0.1 M NaCl, pH 7.5	Sigma-Aldrich	DUO82049	Tris (Caledon Laboratories - 8980-1), NaCl (Caledon - 7560-1)
0.01x Wash Buffer B: 2 mM Tris, 1 mM NaCl, pH 7.5	Sigma-Aldrich	DUO82049	Tris (Caledon Laboratories - 8980-1), NaCl (Caledon - 7560-1)
Duolink In Situ Mounting Medium with DAPI	Sigma-Aldrich	DUO82040	