Immunohistochemistry - Drosophila Embryo

Sonia Hall

Abstract

Citation: Sonia Hall Immunohistochemistry - Drosophila Embryo. **protocols.io**
dx.doi.org/10.17504/protocols.io.cutwwm
Published: 29 Jul 2015

Guidelines

The following is a list of antibody concentrations for use with the protocol 'Immunohistochemistry - Drosophila Embryo'
Custom antibodies were provided by the published authors.

DSHB Antibodies

Antigen	Host Species	Product ID	Concentration	Product Link
DE-Cadherin	rat	DCAD2	1:16	http://dshb.biology.uiowa.edu/cadherin-DE-
Coracle	mouse	50:50 mix of C566.9 and C615.16	1:50	http://dshb.biology.uiowa.edu/Coracle and http://dshb.biology.uiowa.edu/Coracle_2
Engrailed	mouse	4D9	1:10	http://dshb.biology.uiowa.edu/4D9-anti-engrailed-invected
Alpha-spectrin	mouse	3A9	1:16	http://dshb.biology.uiowa.edu/3A9-323-or-M10-2_2
Mmp	mouse	3A6B4	1:20	http://dshb.biology.uiowa.edu/3A6B4
Hindsight	mouse	1G9	1:20	http://dshb.biology.uiowa.edu/hindsight-protein
Atp-alpha	mouse	a5	1:50	http://dshb.biology.uiowa.edu/a5
Nervana	mouse	Nrv5F7	1:50	http://dshb.biology.uiowa.edu/Nervana-protein
Discs Large	mouse	4F3	1:10	http://dshb.biology.uiowa.edu/4F3-anti-discs-large
Fasciclin III	mouse	7G10	1:100	http://dshb.biology.uiowa.edu/7G10-anti-Fasciclin-III_2
Crumbs (concentrate)	mouse	Cq4-c	1:50	http://dshb.biology.uiowa.edu/Crumbs
Armadillo	mouse	N2 7A1	1:50	http://dshb.biology.uiowa.edu/N2-7A1-ARMADILLO
Neuroglian	mouse	BP 104	1:25	http://dshb.biology.uiowa.edu/neuroglian
Flamingo	mouse	Flamingo #74	1:20	http://dshb.biology.uiowa.edu/Flamingo-74
N-Cadherin	rat	DN-Ex #8	1:20	http://dshb.biology.uiowa.edu/cadherin-DN-
Rho1	mouse	p1D9	1:40	http://dshb.biology.uiowa.edu/p1D9-anti-rho1
Alpha-catenin	rat	DCAT-1	1:10	http://dshb.biology.uiowa.edu/catenin-alpha-
Draper	mouse	Draper 8A1	1:25	http://dshb.biology.uiowa.edu/Draper8A1
Delta	mouse	C594.9B	1:50	http://dshb.biology.uiowa.edu/Delta-extracellular-domain
Elav	mouse	ELAV-9F8A9	1:100	http://dshb.biology.uiowa.edu/Elav-9F8A9

Custom Antibodies

Antigen	Host Species	Product ID	Concentration	Citation - Pubmed ID
Pio-Pio	rabbit	Custom	1:100	12973360
Neuroglian (1B7)	mouse	Custom	1:10	2805067
sqh-1P	guinea pig	Custom	1:250	20920606
sqh-2P	rat	Custom	1:500	20920606
Mcr	guinea pig	Custom	1:400	24496625
Contactin	guinea pig	Custom	1:2000	15459097
Kune	rabbit	Custom	1:1000	20407131
Neurexin	rabbit	Custom	1:500	8978610
Uninflatable	guinea pig	Custom	1:400	19818339
Mtf	guinea pig	Custom	1:500	20935638
Squash	mouse	Custom	1:400	20920606

Protocol

Step 1.
Time collection of embryos to obtain the correct stage desired for analysis.

Collection is completed using apple juice agar plates with a small amount of yeast paste applied to surface.

Our collections are typically conducted at 25 degrees C, unless otherwise specified in published materials and methods.

Step 2.
Wash embryos from apple juice agar plate and into an embryo wash basket using a small paintbrush and embryo wash solution.

Step 3.
Place wash basket into a small tray and pour household bleach on top of the embryos until completely covered. Let sit for 3 minutes to remove chorion.

DURATION
00:03:00

Step 4.
Rinse bleach from wash basket and embryos. Once bleach is removed, transfer embryos to container with 50:50 4% paraformaldehyde and heptane.

Shake on orbital rotator at 150rpm for 20 mins

REAGENTS
- Heptane CAS 142-82-5 by Contributed by users

DURATION
00:20:00

Step 5.
Allow solution to heptane and PFA to separate. Remove bottom phase (PFA) and dispose of as required. Be sure to not disturb or remove the embryos that are trapped in the interface.

Add Methanol in equal volume to the remaining heptane. Shake vigorously for 20 seconds and allow embryos to settle to the bottom of the container.

Remove heptane from the top layer and most of the methanol. Add methanol and remove two more times.

Transfer embryos in methanol to disposable culture tubes (flint glass) and allow to settle.

Remove methanol without disturbing the embryos at the bottom of the tube.

REAGENTS
- Disposable Culture Tubes 14-958-A by Contributed by users

Step 6.
Rinse embryos with 750ul of blocking solution, once.

Allow embryos to settle to the bottom of tube.
Remove blocking solution and rinse twice with 1X PBS.
Remove 1X PBS and add 750ul of blocking solution.
Seal tube with parafilm and rock gently for 30 minutes.

DURATION
00:30:00

Step 7.
Remove blocking solution and rinse once with 1X PBS.
Remove 1X PBS and add blocking solution. Add primary antibodies in optimized concentrations. (See Optimized Concentrations for Developmental Studies Hybridoma Bank Antibodies for use with Drosophila Embryos).

Wrap tube with parafilm and rock overnight at 4 degrees Celsius.

Step 8.
Remove blocking solution with primary antibodies.

Rinse three times with 1X PBS. Remove 1X PBS and add 750ul of blocking solution.
Wrap tube with parafilm and rock at room temperature for 30 minutes.

DURATION
00:30:00

Step 9.
Remove blocking solution and rinse once with 1X PBS. Remove 1X PBS and add new blocking solution.
Add secondary antibodies at optimized concentrations.
Our studies used concentrations of 1:1000.

Wrap tube with parafilm and foil. Rock gently for 2-3 hours at room temperature.

DURATION
03:00:00

Step 10.
Remove blocking solution with secondary antibodies. Rinse three times with 1X PBS. Add 750ul of blocking solution.
Wrap tube with parafilm and foil. Rock for 30 minutes.

DURATION
00:30:00

Step 11.
Remove blocking solution and rinse once with 1X PBS.

Carefully remove embryos with a glass pipette that has been rinsed with blocking solution and transfer to a microscope slide.

Using Whatman's paper, carefully wick away excess 1X PBS.

Add 35ul of mounting media and cover with coverslip.
Seal with nail polish if desired.