Western Blot – Glycerol Kinase Ab NBP1-57033

SDS-Page	
1	Assemble the acrylamide gel apparatus. Check with DI water for leaks.
2	Prepare resolving gel; pour immediately after adding TEMED and mixing gently. 12% Resolving, 4% Stacking
3	Pour gel leaving enough room for comb. Add DI or methanol on surface to flatten gel, wait until dry.
4	Prepare stacking gel; pour immediately and add comb, avoiding bubbles. Wait until dry.
5	Prepare protein, 50 ug in a tube. Add protein buffer to 1X. Heat at 37 degrees for 30 minutes.
6	Remove the comb and rinse with the electrophoresis buffer to remove bubbles.
7	Place in electrophoresis tank, fill with buffer to the top.
8	Add 25 uL of sample in protein buffer to each well, include one well for protein markers.
9	Connect Apparatus to circuit, run for 2 hours at 100 volts. Disconnect when dye reaches bottom.

Transfer Blot	
1	Place SDS-Page gel in cold transfer solution for 10 minutes.
2	Cut out membrane, cut right corner for positioning, and soak in cold transfer solution for 10 minutes.
3	Assemble cassette as follows: Fiber Pad, Paper, Membrane, gel, paper, fiber pad where the gel is on the black (negative) side and the membrane is on the clear/red (positive) side.
4	Place cassette into tank and fill with cold transfer buffer. Add a stirring rod and place on ice.
5	Begin stirring and run at 200 mA for 2 hours.

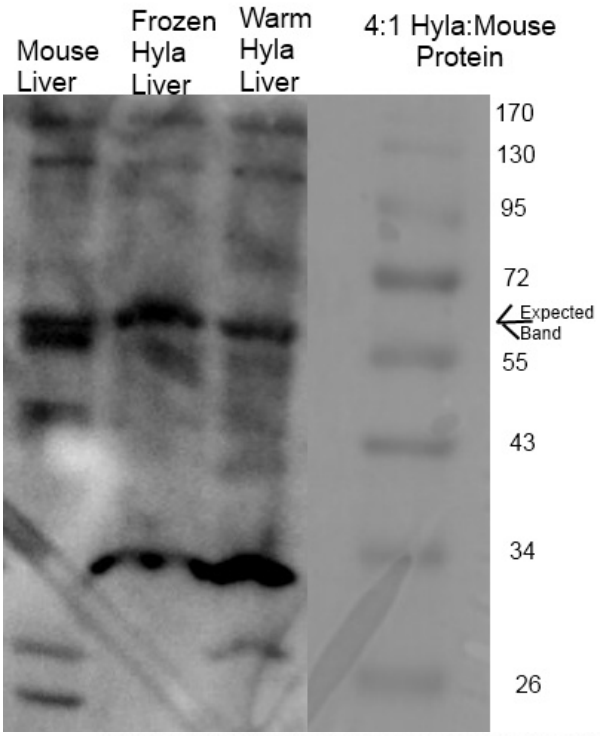
Adding Abs	
1	Rinse with PBST 3 times for 10 minutes each.
2	Remove PBST and put in blocking solution (5% BSA in PBST). Rock for O/N at 4°C
3	Rinse with PBST 3 times for 10 minutes each.
4	Add Primary Ab, 1:1000 in 1% BSA PBST.
5	Incubate for 1.5 hours at room temperature.
6	Rinse with PBST 3 times for 10 minutes each.
7	Prepare 2nd Ab by diluting 1:10,000 in 1% BSA PBST.
8	Apply 2nd Ab for 1.5 hours.
9	Rinse with PBST 3 times for 10 minutes each.

Imaging

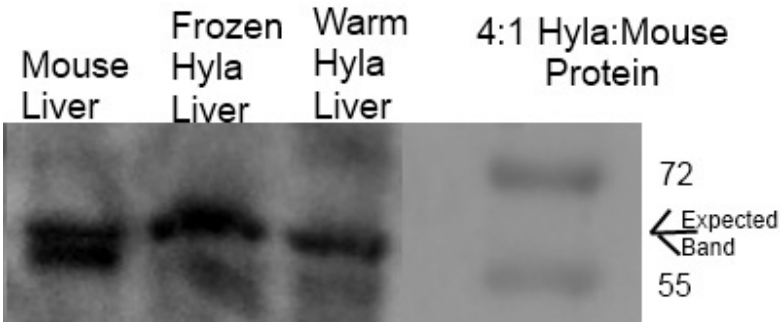
1	Mix Reagents A and B at a 1:1 ratio, making just enough to cover the membrane. Suggested .125 mL per square cm of membrane. Reagent is light sensitive, make immediately prior to use.
2	Remove membrane from PBST and remove moisture w/o touching membrane. Add Mixed reagent.
3	Put membrane in machine wrapped in plastic wrap and picture for chemiluminescence.

Cold Acclimation	Animal brought to 4°C over 2 months before sac.
Warm Acclimation	Left at 20°C.
Frozen	Brought to -2.5°C, sac 24 hours after initiated ice formation.

Glycerol Kinase Western 1:500

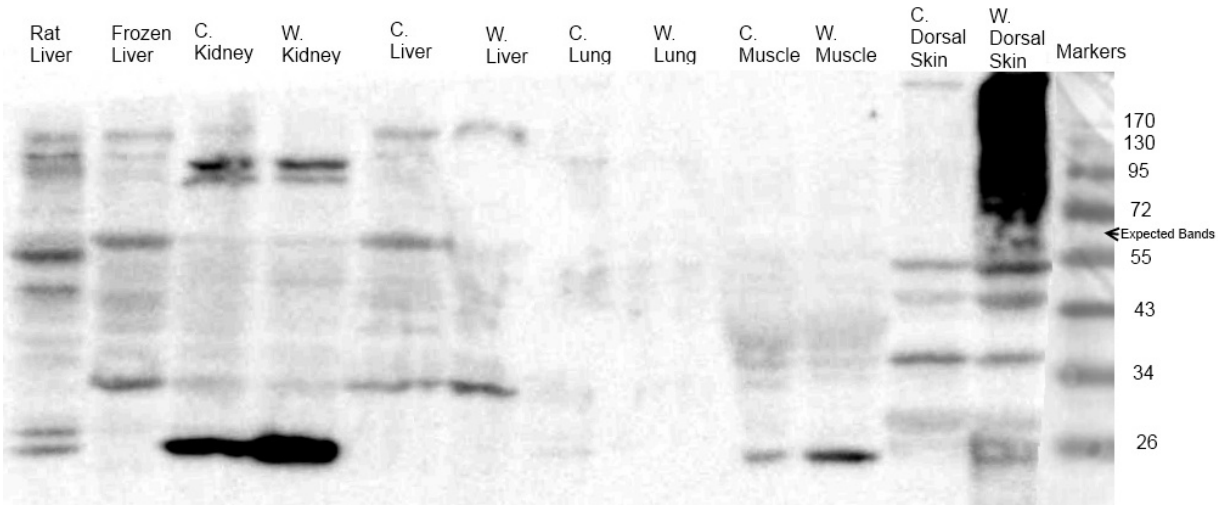


Glycerol Kinase Western 1:500



Glycerol Kinase 1:1000

W. Warm-acclimated Hyla chrysoscelis
C. Cold-acclimated Hyla chrysoscelis



bActin Ab 1:1000

W. Warm-acclimated Hyla chrysoscelis
C. Cold-acclimated Hyla chrysoscelis