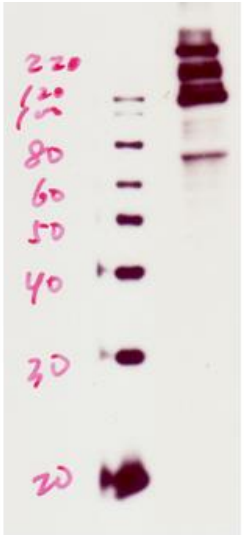
WESTERN BLOT PROTOCOL

Protein Extraction:

All reagents were purchased from Sigma-Aldrich unless otherwise specified. Wash very small tissue with ice-cold PBS and drain off PBS. Transfer the tissue into a 1.5ml centrifuge tube. Add 500µl of ice-cold lysis buffer (1% NP40, 150mM NaCl, 20mM Tris-HCl, 1% Triton X-100, 4mM PMSF, 1:100 Proteinase Inhibitor Cocktail) to the tissue and grind tissue with homogenizer quickly and thoroughly and ultrasonic the mixture at appropriate intervals. Incubate on ice for at least 15 minutes to lyse cells. Centrifuge the lysate at 14,000 x g in a precooled centrifuge for 20 minutes at 4°C. Immediately transfer the supernatant to a fresh centrifuge tube and discard the pellet. Determine the protein concentration by using Bio-Rad Protein Assay Dye Reagent (Cat#500-0006). At this step, the sample can be divided into aliquots and stored at -80ºC.

Western blot procedure:

Prepare 10% SDS-PAGE gels (Reagents from Bio-Rad), load 20µg for each sample and run gel in the Running Buffer (25mM Tris-HCl; 250mM Glycine; 0.1% SDS) around 1.5 hrs. Transfer the membranes in Transfer Buffer (24mM Tris-HCl; 194mM Glycine; 0.01%SDS) at 60V for 2.5 hrs. Stain the membranes with Ponceau S (0.2%) after transfer to make sure the transfer efficiency. Block membranes with 25 ml of 3% milk in TBST Buffer (10mM Tris; 150mM NaCl; 0.1% Tween-20) and incubate at room temperature for 30-60 minutes. Prepare primary antibody against **MYH6** (**NBP2-36746**) at 1:500 with blocking buffer (3% milk in TBST) and rock slowly overnight at 4°C. Wash membranes with TBST 3 time, 5minutes each. Add 6-10 ml of HRP-linked secondary antibodies and rock slowly at RT for 1-1.5 hrs. Wash membranes with TBST 3 time, 5minutes each and detect signal with ECL reagent (HyGOL, Denville Scientific, Cat#E2500) according to the company instruction.



Western Blot: MYH6 Antibody (CL2162) [NBP2-36746] - Human heart tissue lysates