

## Tissue Extracts for GLUT1&4 Western Blots

### Homogenization Buffer (make fresh the day of the experiment)

100 ul 1M TRIS (pH = 7.4)  
40.05 mg NaCl  
500 ul Glycerol  
50 ul PMSF 10 mg/ml MeOH  
200 ul 25x Complete (Roche)  
50 mg Decyl- $\beta$ -maltopyranoside (Sigma D7658)  
QS to 5mls with H<sub>2</sub>O

### Homogenization

- harvest tissue using tongues that are cooled in liquid N<sub>2</sub>
- pulverize tissue using metal tissue pulverizer that is cooled in liquid N<sub>2</sub>
- add 80 ul (2 epitrochlearis muscles) – 500 ul (1 gastrocnemius muscle) buffer to sample (in Eppendorf tube) RIGHT AWAY
- homogenize in Eppendorf tube using pellet pestle RIGHT AWAY
- put samples on ice RIGHT AWAY
- rotate samples 60 min in cold room
- spin 20 min (table top centrifuge; 15,000 rpm @ +4 °C)
- transfer supernatant to fresh tube
- aliquot and store at -80

### Western Blot:

Do **NOT** Boil Samples for GLUT1 / 4 Western Blots

Use 10% gel

10 ug protein for GLUT4, and 50 ug protein for GLUT1

Prepare 1<sup>st</sup> and 2<sup>nd</sup> antibody in 2.5 % milk

Antibodies from Mike Mueckler: GLUT1 1:800

GLUT4 1:2,500

2<sup>nd</sup> antibody for both: anti rabbit 1:4,000

Use 6x Sample Buffer Containing DTT:

3.5 ml 1M TRIS-HCl (pH=6.8)

5.2 ml 20% SDS

3 ml Glycerol

0.93 g DTT

2 mg bromphenol blue

QS to 10 mls with H<sub>2</sub>O

Aliquot in 0.5 mls and store at -20 °C

To make 25x Complete:

Tablets stored at +4 °C

Dissolve tablet in 2ml H<sub>2</sub>O and store at -20 °C for up to 20 days

Expected sizes for Glut 1 and 4 (same size): you will see either a smear or double band at 50 and 45 kDa

