# Skeletal Muscle Protein Carbonylation Assay Protocol

1. Prepare Protein Lysis Buffer by diluting 10X stock Protein Lysis Buffer from Cell Signaling Company (Cat No.9803) in Milli-Q water and added PMSF to the final concentration of 1 mM and DTT to the final concentration of 50 mM from stock solutions of 0.2 M and 1 M, respectively (Need 1 ml Protein Lysis Buffer for each sample).

2. Harvest skeletal muscles (soleus and white vastus lateralis muscles) from mice under anesthesia. Place the muscle immediately in a 5-ml Falcon tube (which contains 1 ml ice-cold Protein Lysis Buffer), and homogenize the muscle sample with the tube in an ice-water bath (a beaker containing ice and water) using an Ultra Turrax T25 Polytron$^{TM}$ homogenizer at the maximal speed for 3 x 10 seconds with 10-second intervals.

3. Transfer the homogenate to a 1.5-ml eppendorf tube and centrifuge by using the Eppendorf Centrifuge at the maximal speed for 5 min at 4 °C.

4. Transfer the supernatant to a 10K NMMWL-0.5 ml Ultrafree Filter Unit (Millipore, Bedford, MA, USA. Cat No. UFV5BGC25) to concentrate the sample to a volume of ~60 µl by using the Eppendorf Centrifuge at the maximal speed at 4°C (about 30 min).

5. Determine the protein concentration by using the BioRad protein concentration assay (Please see protein assay protocol).

6. Transfer homogenate equivalent to 20 µg protein to a new Eppendorf tube and add Protein Lysis Buffer to a final volume of 5 µl. Denature the protein by adding 5 µl of 12% SDS at room temperature. Set a negative control tube with similar amount of protein and SDS.

7. Add 10 µl of 1X DNPH (Included in the kit) to the tube and 10 µl 1X Derivetization-Control Solution to the negative control.

8. Incubate for 15 min at RT (20-25°C).

9. Add 7.5 µl Neutralization Solution to each tube to stop the reaction.

10. Now both the treated sample and the negative control are ready to be loaded on a SDS-PAGE gel (The following procedures are for regular Western blot analysis).

11. Run the samples on an SDS-PAGE gel (100V, 2.5h).

12. Transfer the gel to a PVDF membrane (4°C, overnight).

13. Block the membrane for 1 hr in 5% fat-free milk/PBS-T (TBS-T buffer, 0.1% Triton in PBS).

14. Incubate the blot overnight at 4°C with gentle rocking with the 1° antibody (Provided by the manufacturer) diluted 1:150 in 5% fat-free milk/TBS-T just before use.

13. Rinse the membrane 2 X 5 minutes with 1X TBS-T at RT.

15. Incubate the blot for ~1 hr at RT with gentle rocking with the 2° antibody (Provided by the manufacturer) diluted 1:300 in 5% fat-free milk/TBS-T

16. Rinse the membrane 3 X 5 minutes with 1X TBS-T at RT.

17. Drain the excessive buffer and cover the membrane with ECL Advance™ Western Blotting Detection reagent.

18. Acquire biochemiluminescence image of the Western blot by using the Bio-Rad VersaDoc ™ Imaging System with different exposure times.