# Verification of protein changes by parallel reaction monitoring (PRM)

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PROTOCOL STATUS

**Working**

1 200ug protein was taken from each sample of each group separately, and 10fmol heavy isotope-labeled peptide DSPSAPVNVTVR (Thermo Fisher Scientific Inc.) was incorporated as internal standard.

2 DTT was added to a final concentration of 10 mM, and then heated on the boiling water bath for 15min following cooling it to room temperature.

3 200μL UA buffer (8M Urea, 150mM Tris-HCl, pH8.0) was then mixed in it after centrifuging at 14000 x g for 30min.

4 100μL IAA (50mM IAA in UA) was added with 600rpm shaking for 1min.

5 100μL NH4HCO3 buffer (50mM) was added after centrifuging at 14000 x g for 20min twice.

6 Then 40μL NH4HCO3 buffer (4μg Trypsin) was added after shaking at 600rpm for 1min.

7 40μL NH4HCO3 buffer (50mM) was added after centrifuging at 14000 x g for 30min.

8 The filtrate was collected. The digested peptides were desalted and lyophilized, then reconstituted with 0.1% formic acid, and the peptide concentration was determined by OD280.

9 A chromatographic separation technique was performed by high performance liquid chromatography (HPLC) using Easy nLC that included Buffer A (0.1% Formic acid aqueous solution) and Buffer B (acetonitrile with 0.1% formic acid aqueous solution) (acetonitrile 84%).

10 Samples were added to the trap column and then gradient separated using a Thermo scientific EASY column at rate of 300nl/min. PRM mass spectrometric analysis was undertaken by Q-Exactive HF (Thermo Scientific Inc.).

11 The full scan was taken at a resolution of 60,000 (@m/z 200) with a scan mass range of between 300–1800m/z for 60min.

12 The AGC target was 3e6, the maximum IT was 200ms, and 20 PRM (MS2 scans) were collected according to the inclusion list after each full MS scan.

13 The secondary MS parameters included an isolation window of 1.6Th, resolution of 30,000 (@m/z 200), the AGC target was 3e6, the maximum IT was120 ms, the MS2 activation type was HCD, with normalized collision energy of 27.

14 The generated original file was analyzed using Skyline software version 3.5.0.

15 We manually checked and corrected peak selection according to MS/MS spectra, retention time, the transitions, and mass accuracy.

16 After normalizing the quantitative information by the heavy isotope-labeled peptide, a relative quantitative analysis was performed on the target peptide and the target protein.