Reversible Lysine Derivatization Enables Improved Arg-C (iArg-C) Digestion, a Highly Specific Arg-C Digestion Using Trypsin

Zhen Wu[1], Jichang Huang[1], Jianan Lu[1], Xumin Zhang[*,1]

[1]State Key Laboratory of Genetic Engineering, Department of Biochemistry, School of Life Sciences, Fudan University, Shanghai 200438, China

Supplemental Information

iArg-C Digestion Protocol

Sample preparation TIMING 3 h

1) Cell lysate. Add 20 volumes of lysis buffer (4 M guanidine hydrochloride, 100 mM TEAB) to the collected cells after washing three times with PBS buffer, and sonicate for 6 min (2 s sonication with 5 s intervals).
2) Collection. Collect supernatant via centrifugation at 20,000g for 20 min at 4 °C.
3) Protein determination. Determine the protein concentration using Bradford assay.
4) Reduction. Add 10 mM DTT and then incubate at 37 °C for 45 min.
5) Alkylation. Add 100 mM acrylamide and incubate for 1 h at room temperature.
6) Quenching. Add DTT to a final concentration of 50 mM to quench unreacted acrylamide.
7) Dilute the proteins to 1 mg/mL with lysis buffer.

Citraconylation TIMING 2 h

8) Add 5 μL of 2M citraconic anhydride to 200 μL protein sample, and then immediately add 5 μL 4M NaOH. After short vortex, incubate the sample for 10 min at room temperature.
9) Repeat step 8 for another four times to reach a final concentration of 200 mM citraconic anhydride.
10) Incubate the solution at room temperature for 1 h.

Digestion TIMING 15-18 h

11) Buffer displacement. Transfer samples from Eppendorf tubes to Microcon YM-10 filters and centrifuged at 13,800 g for three-time buffer displacement with 100 mM TEAB (pH 8.0). This step takes 3 to 6 h depending on sample

volume and protein concentration.

12) Digestion. Add digestion buffer (100 mM TEAB, pH 8.0) to reach the protein concentration of 1 mg/mL. Add trypsin at a ratio of enzyme/protein as 1:50 and incubate at 37 °C for 12 h.

Sample collection TIMING 3-5 h

13) After digestion, collect the filtrate via centrifugation at 13,800g. To minimize sample loss, wash the filter twice with 10% ACN and collect the filtrates via centrifugation at 13,800g. Pool the three filtrates together.

14) Remove the remaining ACN and concentrate sample to about 1 mg/mL by a Speedvac.

Decitraconylation TIMING 2 h

15) Decitraconylation. Add TFA to a final concentration of 1% (v/v) and incubate at room temperature for 2 h.