Protein extraction, alkylation, and digestion for LC/MS of HEK-293

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Abstract

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Protocol

Step 1.

The HEK-293 proteins were extracted using the standard protocol for the RIPA buffer (NACALAI TESQUE, INC., Kyoto, Japan). Approximately 10^6 harvested cells were washed once in Krebs-Ringer-Buffer (KRB; 154 mM NaCl, 5.6 mM KCl, 5.5 mM glucose, 20.1 mM HEPES pH 7.4, 25 mM $NaHCO_3$). They were resuspended in 30 µl of RIPA buffer, passed in and out through 21G needles for destruction, and incubated on ice for 1 h. They were then centrifuged at 10,000 *g* for 10 min at 4°C, followed by collection of the supernatants; the proteins were quantified by using a Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, U.S.A.).

Step 2.

Further processing was performed using XL-Tryp Kit Direct Digestion (APRO SCIENCE, Naruto, Japan). The samples were solidified in acrylamide gel, washed twice in ultrapure water, washed three times in dehydration solution, and dried. The samples were then processed using an In-Gel R-CAM Kit (APRO SCIENCE, Naruto, Japan). The samples were reduced for 2 h at 37°C, alkylated for 30 min at room temperature, washed five times with ultrapure water, washed twice with destaining solution, and then dried. The resultant samples were trypsinized overnight at 35°C.

Step 3.

The next day, the dissolved digested peptides were collected by ZipTipC18 (Merck Millipore, Corp., Billerica, U.S.A.). The tips were dampened with acetonitrile twice and equilibrated twice with 0.1% trifluoroacetic acid. The peptides were collected by 20 cycles of aspiration and dispensing, washed twice with 0.1% trifluoroacetic acid, and eluted by 0.1% trifluoroacetic acid /50% acetonitrile with aspiration and dispensing five times × three tips followed by vacuum drying. The finalized samples were stored at −20°C.

Step 4.

Before performing LC/MS, they were resuspended in 0.1% formic acid, and the amounts were quantified by Pierce Quantitative Colorimetric Peptide Assay (Thermo Fisher Scientific, Waltham, U.S.A.).