

x 个样本 IP-MS 鉴定报告

项目编号:

客户单位:

联系人:

联系方式

胡志伟

手机号码: 18667909633

河南臻质生物科技有限公司

河南省郑州市金水区鸿苑路 65 号金科智汇谷 1 号楼 2 层

目 录

一、项目总览	2
二、材料与方法	3
1. 样品制备	3
1.1 材料与试剂	3
1.2 蛋白提取	3
1.3 抗体偶联磁珠	4
1.4 免疫共沉淀	4
1.5 胶内酶解	4
2.液相色谱-质谱联用分析	5
2.1 材料与试剂	5
2.2 液质联用参数设置	5
2.3 数据搜库	6
三、英文版实验方法模板（供参考）	6
1. Immunoprecipitation	6
2. In-gel Digestion	7
3. LC-MS/MS Analysis	7
3. Data Processing	8

一、项目总览

本项目结合 IP 实验, 蛋白凝胶电泳, 胶内酶解, 高分辨生物质谱技术 Thermo

Scientific Orbitrap Exploris 480 和数据库搜索，对 x 个细胞样本：以及对应的两个 IgG 样品 进行分析鉴定，详见 Excel 表。数据库使用：Uniprot_x。

鉴定结果详细信息请参见附件中对应的 Excel 表格文件，单蛋白修饰位点鉴定，会提供原始数据和 Excel。

二、材料与方法

1. 样品制备

1.1 材料与试剂

试剂名称	供应商
胰蛋白酶 (trypsin)	Promega
乙腈 (acetonitrile)	Fisher Chemical
甲酸 (formic acid)	Fluka
碳酸氢铵 (NH_4HCO_3)	Sigma
二硫苏糖醇 (dithiothreitol)	Sigma
碘代乙酰胺 (iodoacetamide)	Sigma
IP裂解液	Beyotime
PBS	Biosharp
BCA试剂盒	Beyotime

1.2 蛋白提取

用 PBS 洗涤组织，随后添加组织 3 倍体积的裂解缓冲液使用磁珠研磨组织，随后进行超声破碎细胞，冰上孵育 30min。离心取上清转移到新的 EP 管中，测定蛋白浓度。

1.3 抗体偶联磁珠

涡旋磁珠原液，取磁珠悬液至 EP 管，MPC 去上清，用 1mL PBST 洗涤磁珠，用 500uL PBS 重悬磁珠。随后加入足够量的抗体进行免疫沉淀，室温(18-22°C)旋转 1h。下一步 MPC 去上清，1mL PBS 洗涤四次。

1.4 免疫共沉淀

向细胞裂解液样品中加入抗体结合珠，4°C 旋转过夜后用 PBST 洗涤干净，最后一次洗涤时转移珠子至新的管中，去上清，加入 protein loading buffer 进行蛋白洗脱，洗脱样本进行 SDS-PAGE，取一块后续进行转膜随后开展 WB 实验，一块使用 R250 考马斯亮蓝染色 0.5h 后，进行胶条检测。

1.5 胶内酶解

将胶条切碎，使用含 50 mM 碳酸氢铵 (NH_4HCO_3) 的 50% 乙腈 (acetonitrile) 将胶块脱色。利用 100% 乙腈孵育 5 分钟将胶块脱水，之后移去体系中液相，加入终浓度为 10 mM 的二硫苏糖醇 (dithiothreitol) 溶液，37 °C 孵育 60 分钟。再次使用 100% 乙腈孵育脱水，移去液相后加入终浓度为 55 mM 的碘代乙酰胺 (iodoacetamide)，室温避光孵育 45 分钟。之后使用终浓度为 50 mM 的碳酸氢铵清洗，再次使用 100% 乙腈孵育脱水。最后使用含有 10 ng/ μl 胰蛋白酶 (trypsin) 的 50 mM 碳酸氢铵重悬胶块，冰上孵育 1 小时。除去样品中多余的溶液后，将胶块在 37°C 酶解过夜。酶解后的肽段依次使用 50% 乙腈/5% 甲酸和 100% 乙腈提取出胶块，肽段溶液冷冻旋干后备用。

2.液相色谱-质谱联用分析

2.1 材料与试剂

试剂名称	供应商
超纯水 (H ₂ O)	Fisher Chemical
乙腈 (acetonitrile)	Fisher Chemical
甲酸 (formic acid)	Fluka

质谱仪: Thermo Scientific Orbitrap Exploris 480

2.2 液质联用参数设置

肽段用流动相 A 溶解后, 经 EASY-nLC 1200 超高效液相系统进行分离。流动相 A 为含 0.1%甲酸和 2%乙腈的水溶液, 流动相 B 为含 0.1%甲酸和 80%乙腈的水溶液。液相梯度总共 65 min 设置为:40 分钟 5%~34% B 相(80% ACN, 0.1% FA); 5 分钟 34%~38% B 相; 10 分钟 38%~90% B 相, 10 分钟 90% B 相,流速维持在 300 nL/分钟。

肽段经由超高效液相系统分离后被注入 NSI 离子源中进行电离然后进 Thermo Scientific Orbitrap Exploris 480 质谱进行分析。离子源电压设置为 2.0 kV, 肽段母离子及其二级碎片都使用高分辨的 Orbitrap 进行检测和分析。一级质谱扫描范围设置为 350-1800 m/z, 扫描分辨率设置为 70,000; Orbitrap 扫描分辨率设置为 17,500。数据采集模式使用数据依赖型扫描 (DDA) 程序, 即在一级扫描后选择信号强度最高的前 20 个肽段母离子依次进入 HCD 碰撞池使用 28%的破碎能量进行碎裂, 同样依次进行二级质谱分析。为了提高质谱的有效利用率, 自动增益控制 (AGC) 设置为 5E4, 信号阈值设置为 1E4, 最大注入时间设置为 100 ms, 串联质谱扫描的动态排除时间设置为 15 秒以减少母离子的重复扫描。

2.3 数据搜库

二级质谱数据使用 Proteome Discoverer 2.4 进行检索。检索参数设置如下：数据库设置为 *Uniprot_Mus musculus*；酶切方式设置为 Trypsin/P；一级母离子质量误差容忍度设置为 10 ppm；二级碎片离子的质量误差容忍度设置为 0.02 Da；固定修饰设置为半胱氨酸烷基化。

三、英文版实验方法模板（供参考）

1. Immunoprecipitation

The tissue was washed with PBS, followed by the addition of lysis buffer in three times the volume of the tissue. The tissue was then homogenized using bead milling, and subsequently subjected to sonication to disrupt the cells. The mixture was incubated on ice for 30 min, followed by centrifugation to collect the supernatant. The supernatant was transferred to a new EP tube, and the protein concentration was determined.. Vortex magnetic bead stock solution, transfer bead suspension to EP tube, remove supernatant with MPC, wash beads with 1 mL PBST, and resuspend beads in 500 μ L PBS. Add sufficient antibody for immunoprecipitation and rotate at room temperature (18-22° C) for 1 hour. Next, remove supernatant using MPC, wash four times with 1 mL PBS. Add antibody-coated beads to the cell lysate sample, incubate overnight at 4° C with rotation, then wash thoroughly with PBST. For the final wash, transfer beads to a fresh tube, remove supernatant, and elute proteins with protein loading buffer. Run eluted samples on SDS-PAGE. Transfer one gel for subsequent Western blot analysis. Stain another gel with R250 Coomassie Brilliant Blue for 0.5 hours, then perform gel strip detection.

2. In-gel Digestion

For in-gel tryptic digestion, gel pieces were destained in 50 mM NH_4HCO_3 in 50% acetonitrile (v/v) until clear. Gel pieces were dehydrated with 100 μl of 100% acetonitrile for 5 min, the liquid removed, and the gel pieces rehydrated in 10 mM dithiothreitol and incubated at 56 °C for 60 min. Gel pieces were again dehydrated in 100% acetonitrile, liquid was removed and gel pieces were rehydrated with 50 mM iodoacetamide. Samples were incubated at room temperature, in the dark for 45 min. Gel pieces were washed with 50 mM NH_4HCO_3 and dehydrated with 100% acetonitrile. Gel pieces were rehydrated with 10 ng/ μl trypsin resuspended in 50 mM NH_4HCO_3 and 5 mM CaCl_2 on ice for 10 min. Excess liquid was removed and gel pieces were digested with trypsin at 37 °C overnight. Peptides were extracted with 50% acetonitrile /5% formic acid, followed by 100% acetonitrile. Peptides were dried to completion and resuspended in 2% acetonitrile/0.1% formic acid.

3. LC-MS/MS Analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 μm i.d.). The gradient was comprised of an increase from 5% to 34% solvent B (0.1% formic acid in 80% acetonitrile) over 40 min, 34% to 38% in 5 min , 38% to 90% in 10 min and then holding at 90% for the last 10 min, all at a constant flow rate of 300 nl/min on an EASY-nLC 1200 UPLC system.

The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Thermo Scientific Orbitrap Exploris 480 coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments

were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0s dynamic exclusion. Automatic gain control (AGC) was set at 5E4.

3. Data Processing

The resulting MS/MS data were processed using Proteome Discoverer 2.4. Tandem mass spectra were searched against *Uniprot _Mus musculus*. Trypsin/P was specified as cleavage enzyme. Mass error was set to 10 ppm for precursor ions and 0.02 Da for fragment ions.