小分子-靶点蛋白富集及质谱筛选 (LFQ)

SM-PD Enrichment Kit (Biotin tag, MS grade)

Catalog Number: MG05

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Workflow of SM-PD Enrichment Kit (Biotin tag, MS grade)
Catalog Number: #MG05

PART 1 Guide for large-scale cell culture, protein extraction & Preclear

Culture cells in 10 cm dishes

1. Collect cell pellets
2. Liquid Nitrogen snap-freezing
3. Store at -80°C

1. Extract proteins with **MG05-1**
2. Filter protein solution
3. Protein quantitation (Imultiomics, #MGR02)

Protein preclear with **MG05-5**

Store at -80°C or go to downstream applications

PART 2 Probe concentration optimization

Start at least 21 mg
Protein sample
(**MG05-1** extracted)

1. Preclear with **MG05-5**
2. Divide into **5** parts

Perform SM-binding reactions with **MG05-4** and **Probe**

Perform pull-down with **MG05-5**

Wash with **MG05-2 & MG05-3**

Elute with **MG05-6**

SDS-PAGE & staining (Imultiomics, #MGR01)

1. Obtain optimized **Probe** concentration
2. Obtain optimized **Protein input**/reaction (**X** mg)
3. Obtain optimized **MG05-5** input/reaction (μl)

***Probe**: Biotin-labeled SM (**User Provide**)

PART 3 SM concentration optimization

at least 6× Protein input (**X** mg)
Protein sample
(**MG05-1** extracted)

1. Preclear with **MG05-5**
2. Divide into **6** parts

Perform SM-binding reactions with **MG05-4**, **SM**, and **optimized Probe**

Perform pull-down with optimized volume (μl) of **MG05-5**

Wash with **MG05-2 & MG05-3**

Elute with **MG05-6**

SDS-PAGE & staining (Imultiomics, #MGR01)

Obtain optimized **SM** concentration

***SM**: parent compound (**User Provide**)

PART 4 SM-PD sample preparation for MS detection

at least 3× Protein input (**X** mg)
Protein sample
(**MG05-1** extracted)

1. Preclear with **MG05-5**
2. Divide into **3** parts

Perform SM-binding reactions with **optimized Probe & SM**

Perform pull-down with optimized volume (μl) of **MG05-5**

Wash with **MG05-2 & MG05-3**

On-beads trypsin digestion (Imultiomics, #MG04)

LC-MS detection & LFQ quantification

Statistical analysis to distinguish **SM-targeted Proteins**

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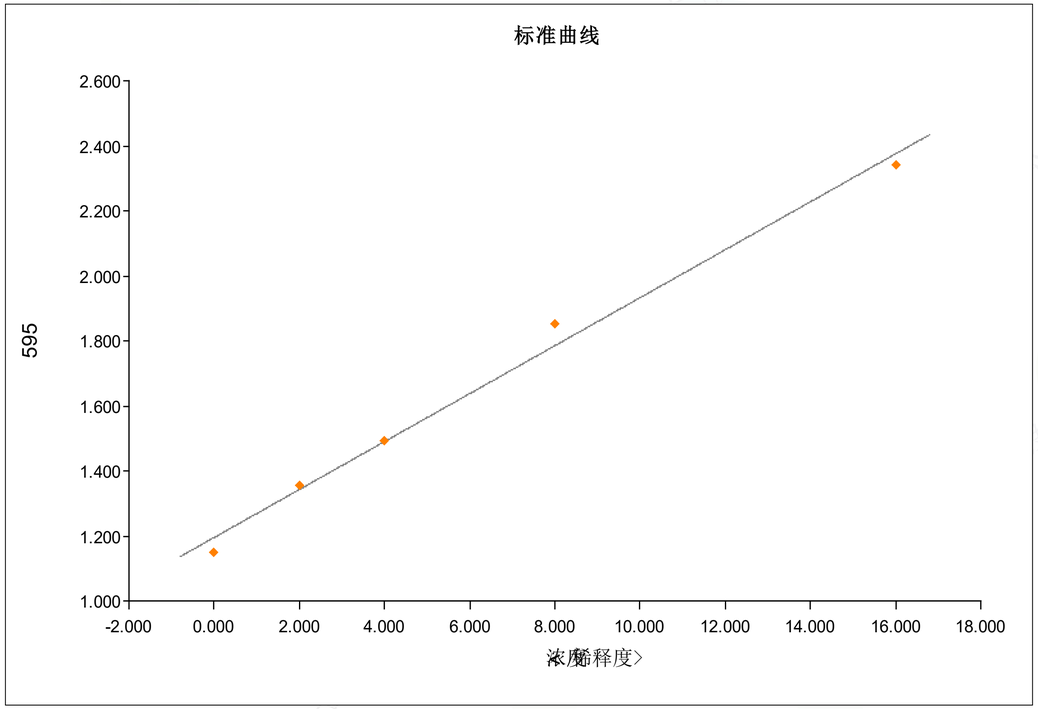
A. Protein extraction, Quantitation & Preclear



曲线名称	曲线公式	A	B	R2
标准曲线	Y=A*X+B	0.0738	1.2	0.991

1. Cell pellet of 7×10cm dishes were lysed with 7×600µl of MG05-1 (Cell lysis buffer).
2. Protein concentration (6.8 mg/ml) was quantified using Bradford Protein Assay Kit (Imultiomics, #MGR02).
3. Protein sample was precleared with MG05-5 according to the protocol of MG05 Kit

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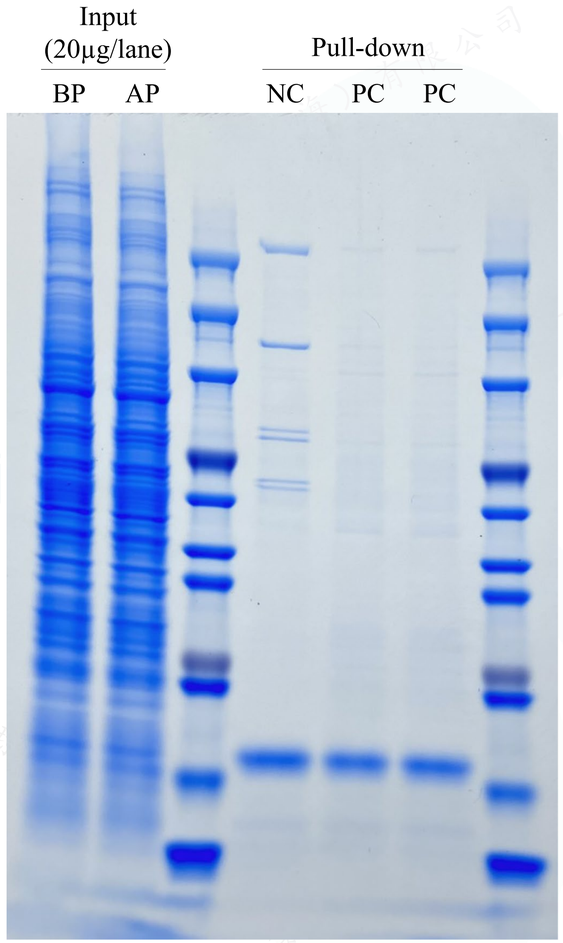
B. Pull-down data of PC in MG05 Kit



1. BP: Before Preclear
2. AP: After Preclear
3. NC: negative control (MG05-5 beads only)
4. PC (Positive control): MG05-4 in MG05 Kit. PC is the biotin-labeled small molecule (SM). The pull-down data of PC showed differential bands in contrast to that of NC.
5. SDS-PAGE gel was stained with Coomassie Blue Staining Reagent (Imultiomics, #MGR01)

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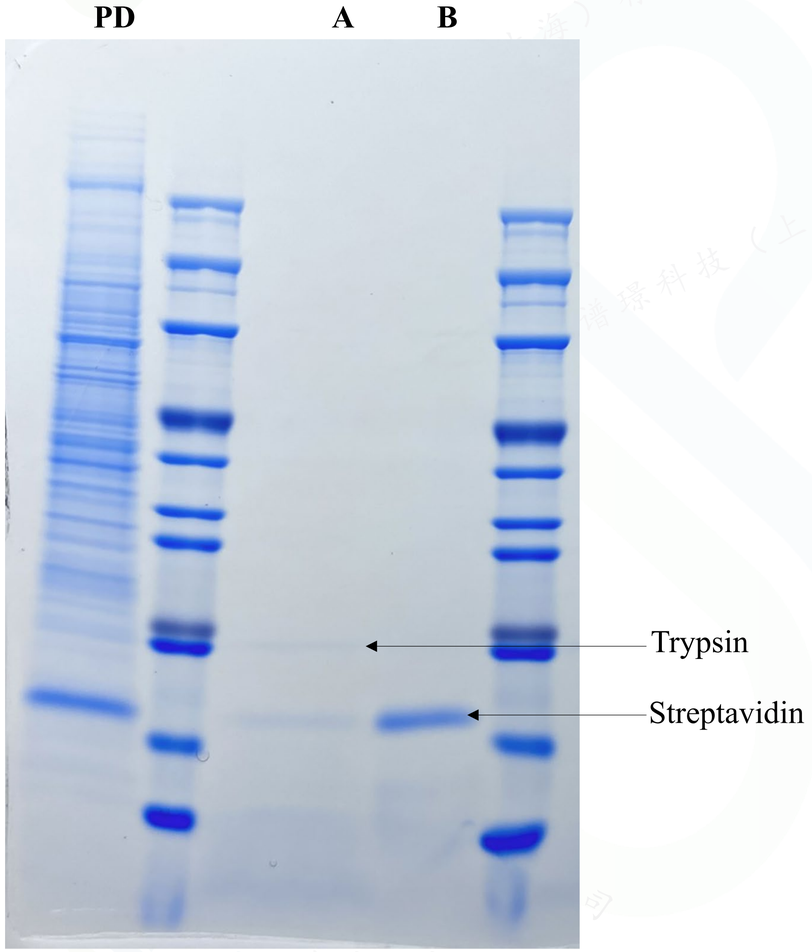
C. Streptavidin remained on beads after on-beads digestion



PD: MG05-5 (Streptavidin-beads) enriched proteins by pull-down. PD was conducted in duplicate.

A. PD sample was carried out on-beads digestion using Co-IP/pull-down in-solution digestion Kit (Imultiomics, #MG04), the enriched proteins were completely digested into peptides in contrast to that of PD.

B. The left beads after on-beads digestion was treated with MG05-6 (5× sample loading buffer) for Coomassie blue staining (Imultiomics, #MGR01). The data showed most of streptavidin remained on beads (MG05-5) after on-beads digestion, less interference of Pull-down enriched proteins identification by LC-MS!

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