

小分子-靶点蛋白富集及质谱筛选 (LFQ) SM-PD Enrichment Kit (Azide tag, MS grade) **Catalog Number: MG07**





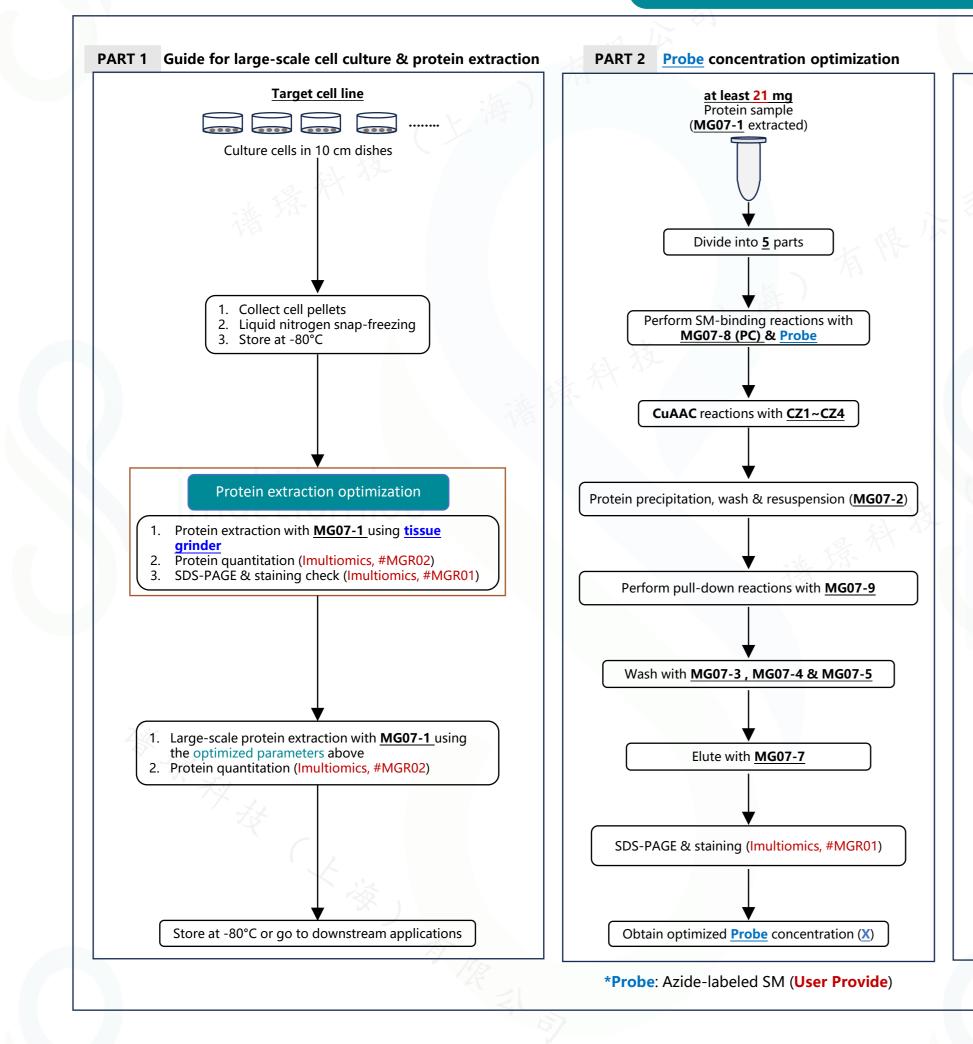


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multiomics

Workflow of SM-PD Enrichment Kit (Azide tag, MS grade) Catalog Number: #MG07



imultiomics

PART 3 SM concentration optimization PART 4 SM-PD sample Preparation for WB & MS at least 25 mg <u>at least <mark>25</mark> mg</u> Protein sample Protein sample (MG07-1 extracted) (MG07-1 extracted) Divide into <u>6</u> parts (NC, Probe, SM in duplicate) (NC-1, Probe-1, SM-1) (NC-2, Probe-2, SM-2) Divide into <u>6</u> parts Perform SM-binding reactions with Perform SM-binding reactions with optimized Probe & SM MG07-8 (PC), optimized Probe & SM CuAAC reaction with CZ1~CZ4 CuAAC reaction with CZ1~CZ4 Protein precipitation, wash & resuspension (MG07-2) Protein precipitation, wash & resuspension (MG07-2) Perform pull-down reactions with MG07-9 Perform pull-down reactions with MG07-9 Wash with MG07-3 , MG07-4 & MG07-5 Wash with MG07-3 , MG07-4 & MG07-5 NC-1, Probe-1, SM-1 NC-2, Probe-2, SM-2 Elute with MG07-7 On-beads trypsin digestion (Imultiomics, #MG04) Elute with MG07-7 LC-MS detection & LFQ quantification SDS-PAGE & staining (Imultiomics, #MGR01) Statistical analysis to distinguish Western blot detection with MG07-6 **SM-targeted Proteins** Obtain optimized <u>SM</u> concentration (Y) *SM: parent compound (User Provide)

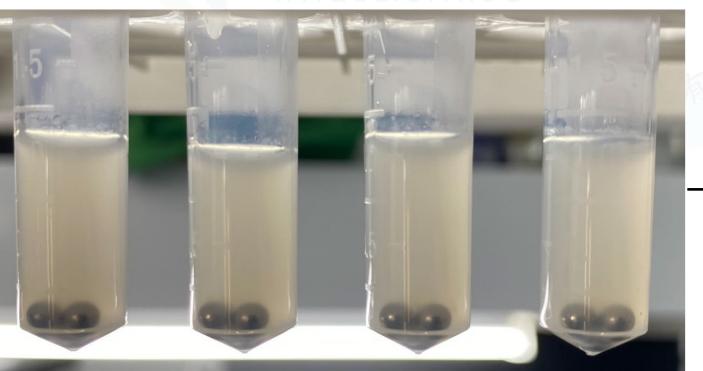
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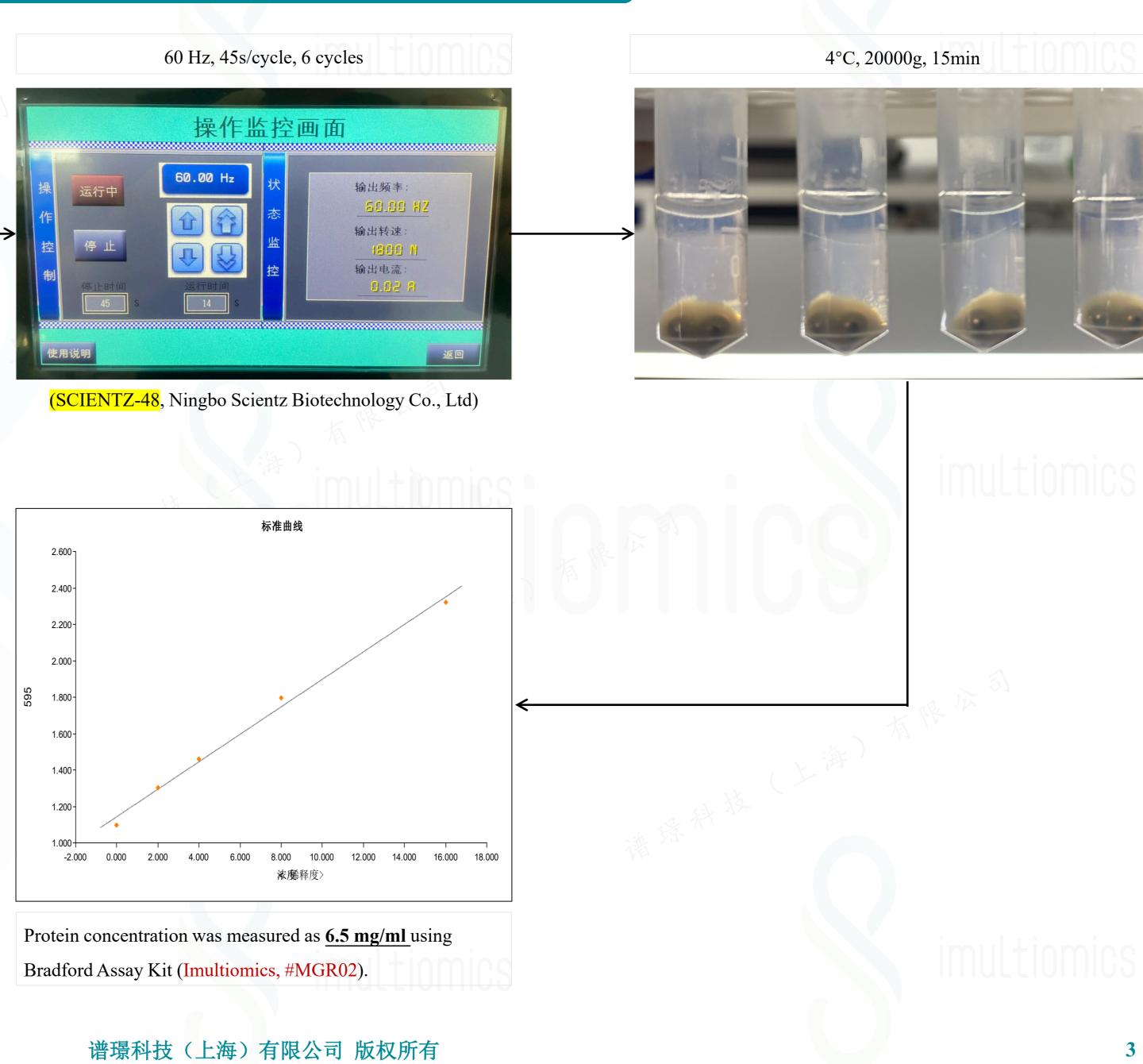
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2

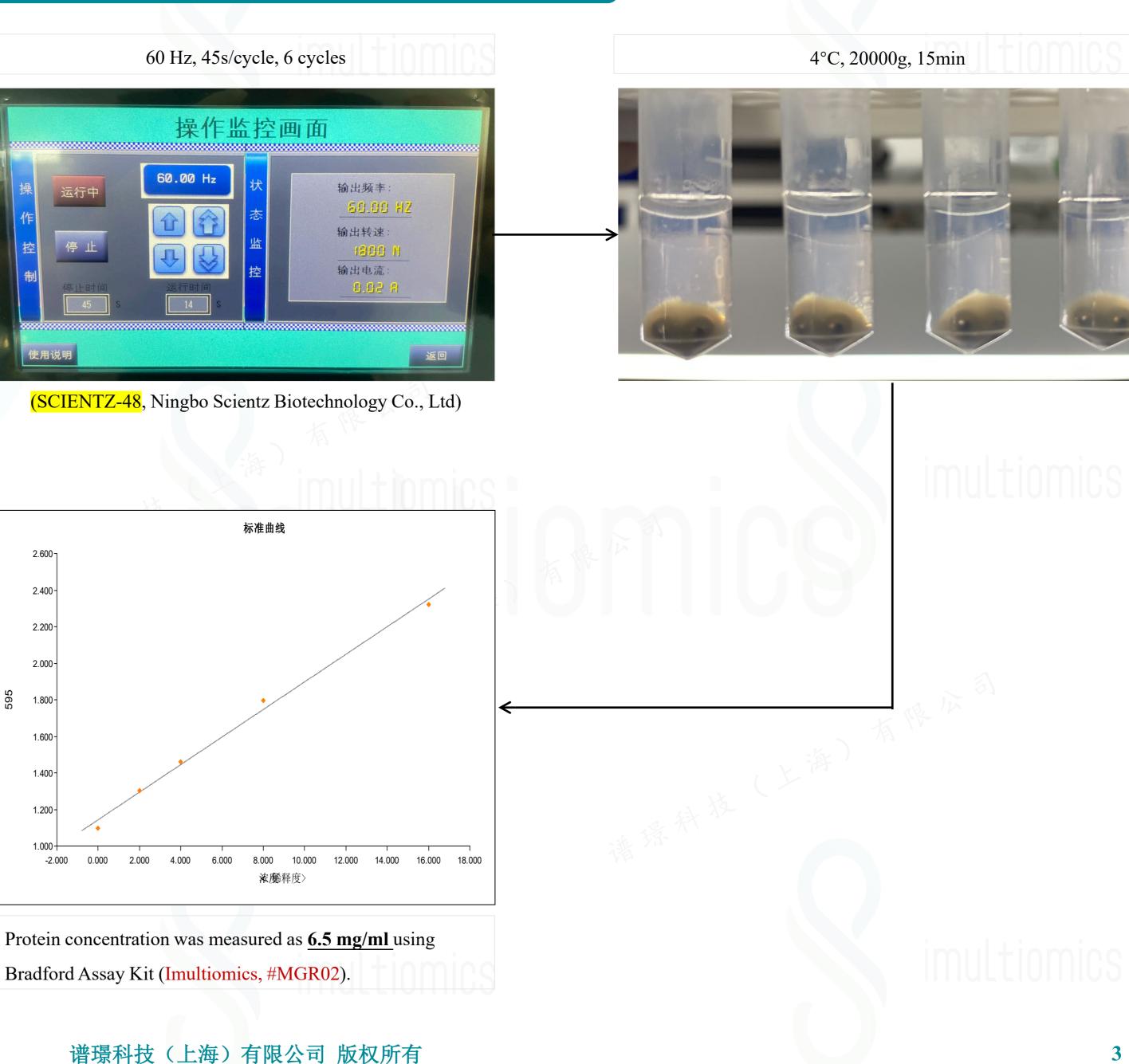
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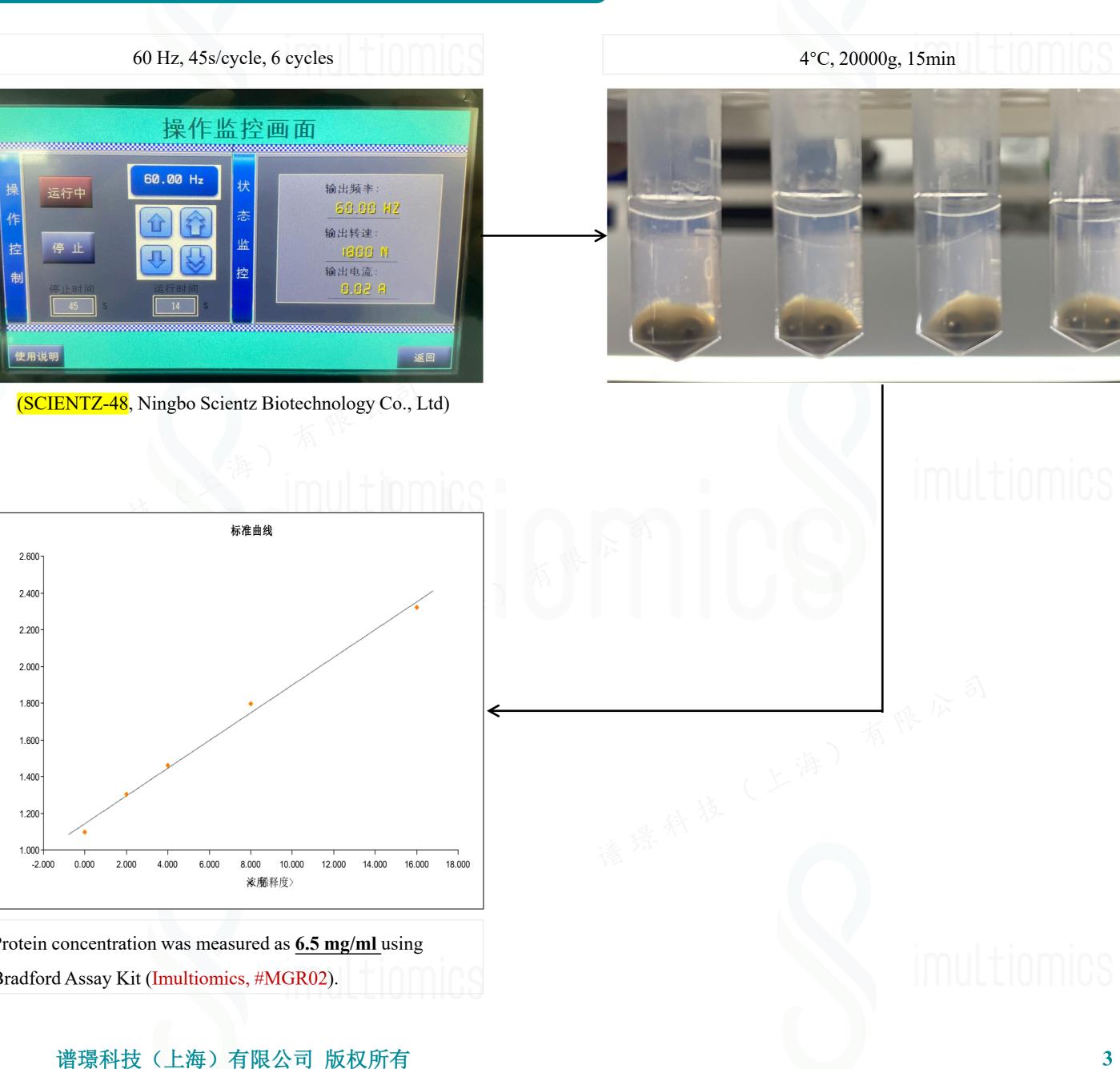
A. Protein extraction & quantitation





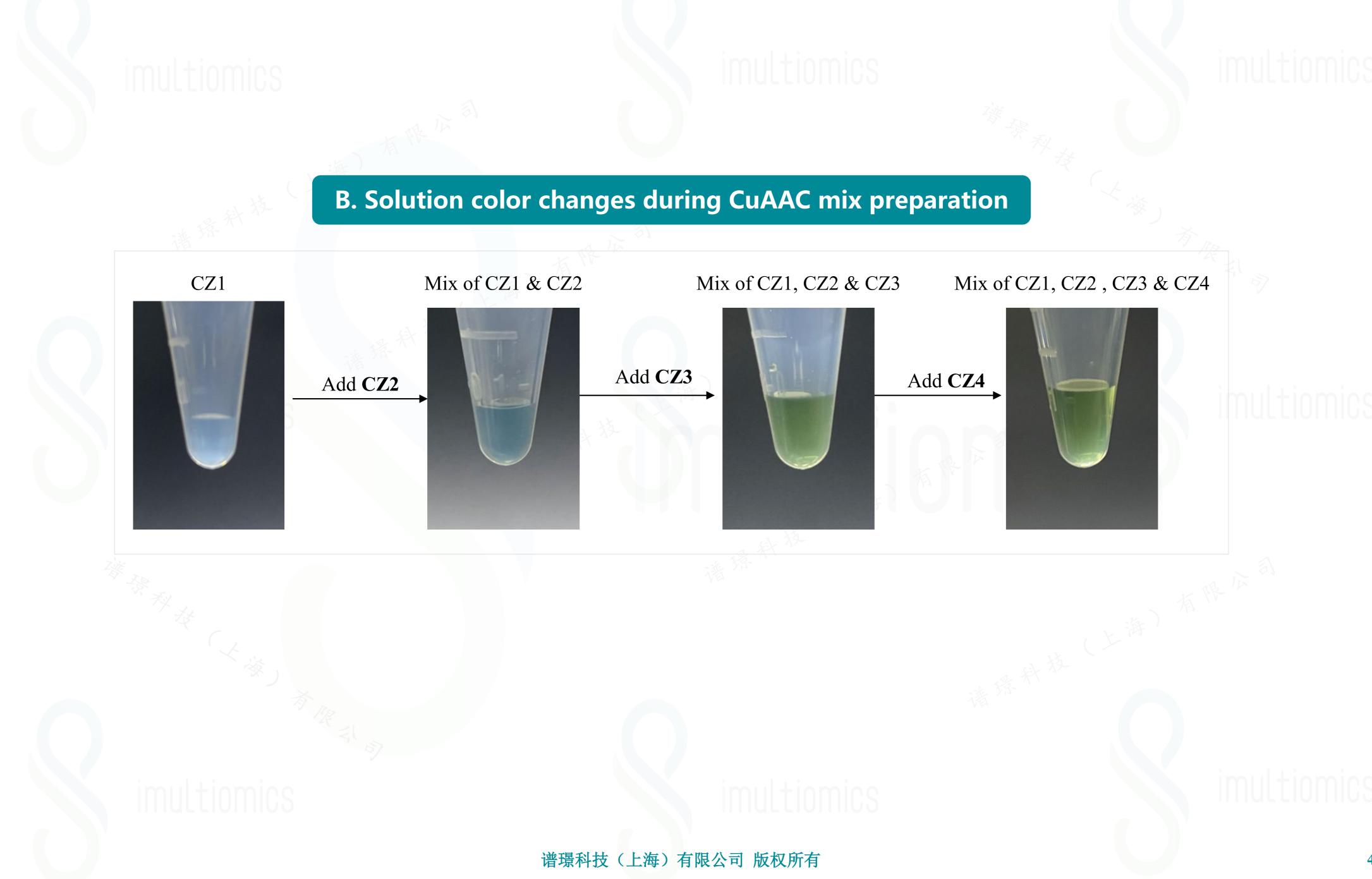
Cell pellet of 9 \times 10 cm dishes was resuspended into <u>3.6mL</u> of MG07-1 (Cell lysis buffer) in #MG07 Kit, allocated to 2ml tubes, and 3×3mm (diameter) of zirconia balls were added into each tubes.









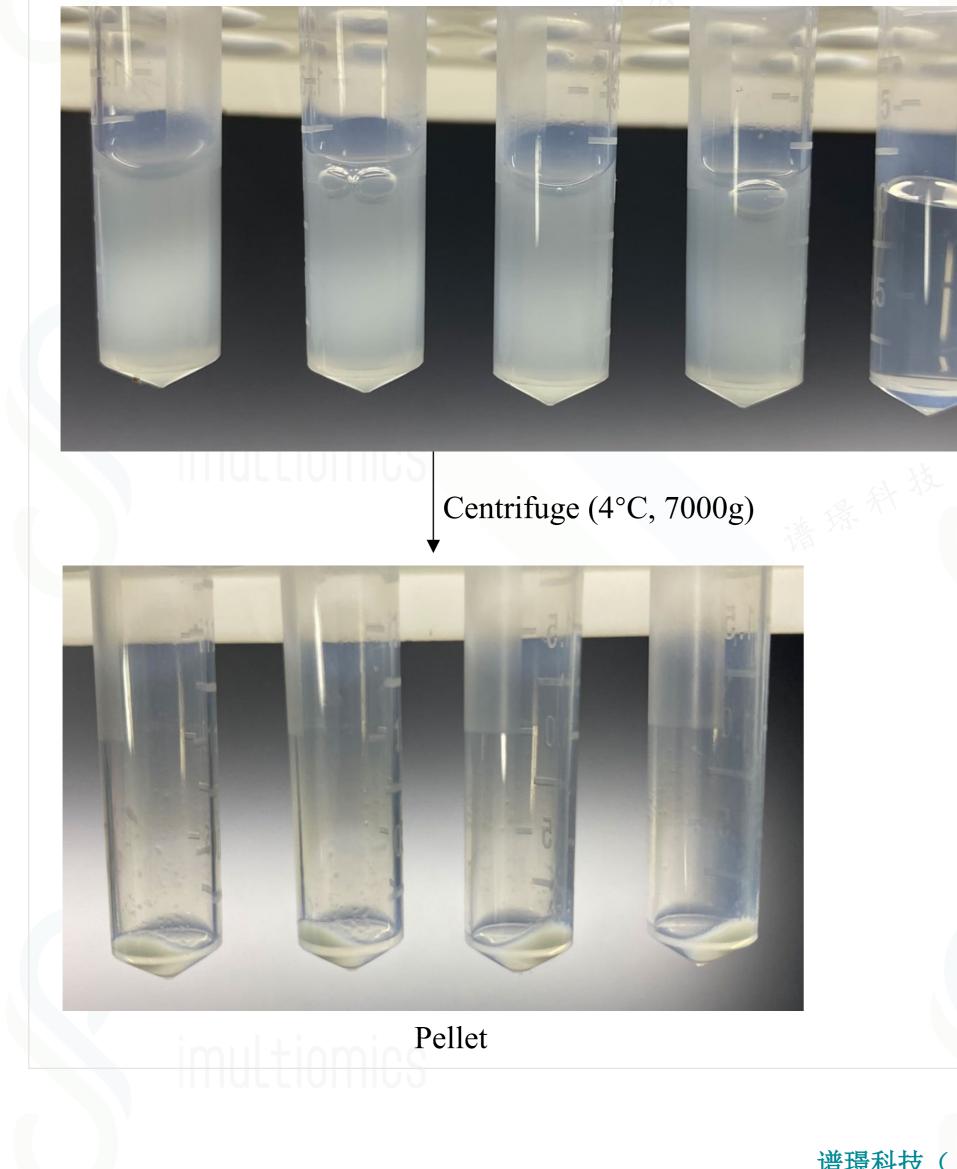




C. CuAAC reaction in Protein samples

Addition of CuAAC mix

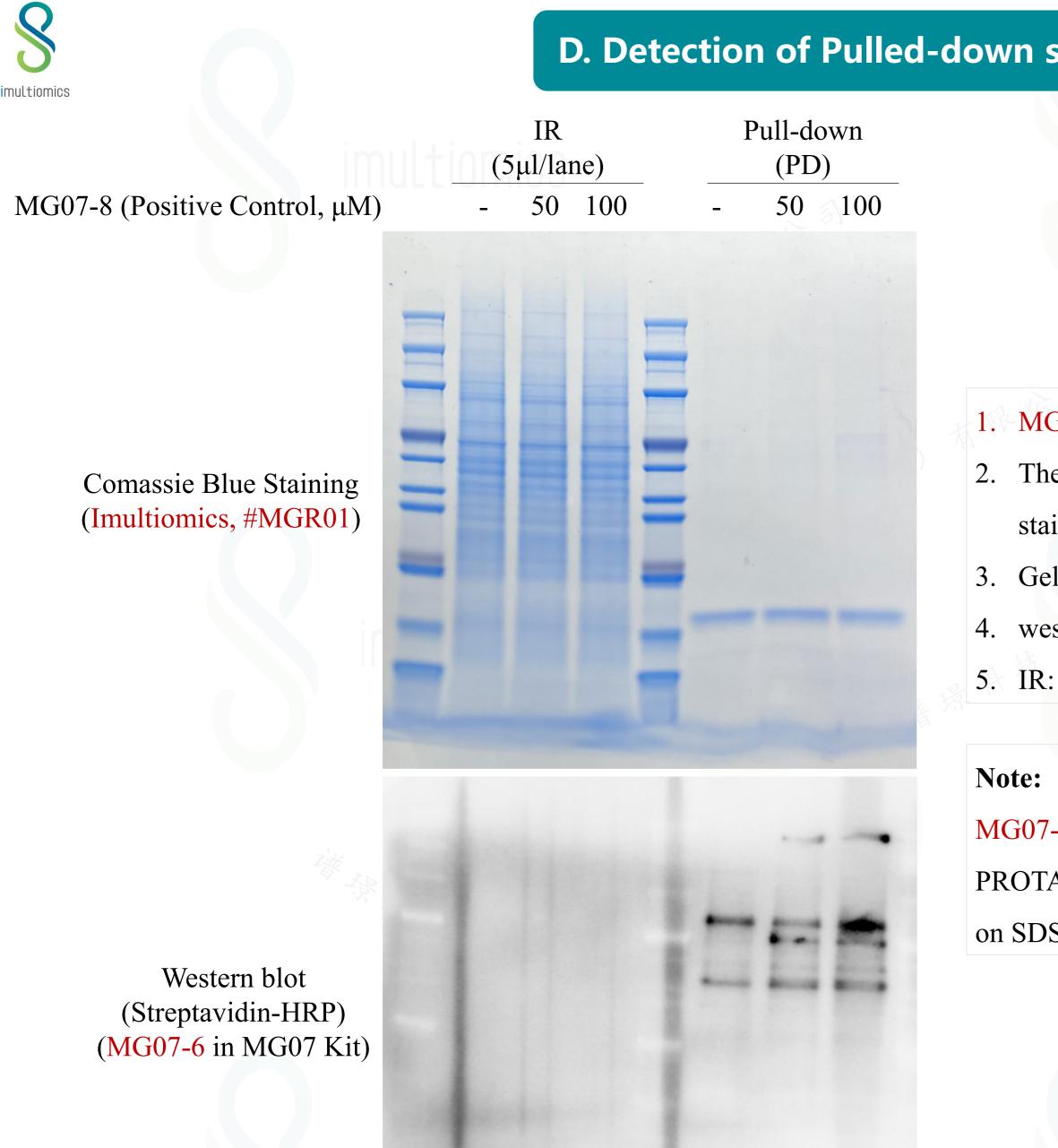




- 1. Protein samples turned cloudy after incubation with CuAAC mix for 1 hour at RT.
- 2. Pellets were collected after centrifuge for downstream pull-down procedure.

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D. Detection of Pulled-down samples by Gel staining & western blot

MG07-8 (Positive Control, PC) in <u>#MG07 Kit</u>, the Azide-labeled SM.

The pulled-down samples of MG07-8 (50, 100µM) using <u># MG07 Kit</u> were detected by gel staining and western blot.

Gel staining (up) showed the enriched protein bands compared to NC (negative control) sample. 4. western blot (down) data was consistent with that of gel staining.

IR: Input of Resuspension Protein

MG07-8 is an azide-labeled SM specific targeting cereblon (CRBN) protein widely used in PROTAC (Proteolysis targeting chimera) system. Therefore, the abundance and pattern of protein on SDS-PAGE/western blot enriched by **#MG07 Kit** may be cell/tissue type-dependent.

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