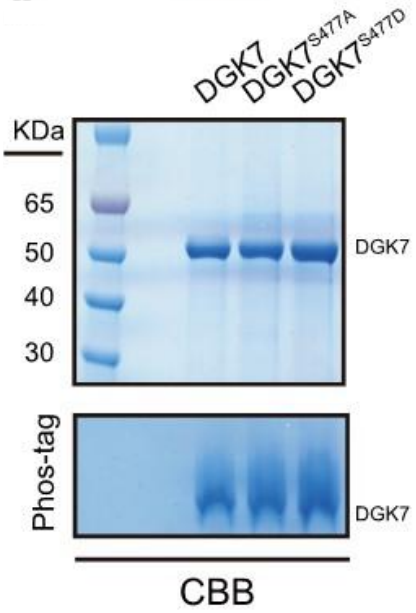
Phosbind Application Case Library (Cat. No. F4002)

(Including SDS-PAGE-Mass Spectrometry Operation Guide)

The following reference cases and SDS-PAGE mass spectrometry operation guide are compiled and organized from literature-reported data, classified across five dimensions: sample type (animal, plant, insect, and microorganisms), experimental system (in vitro kinase reaction, cell lysate, tissue homogenate, purified protein), protein characteristics (single-band protein, multi-band protein, site-mutation modified protein, and high-molecular-weight protein), detection method (tag antibody and target protein non-phosphorylated antibody), and downstream analysis (CBB staining, silver staining, ECL detection, 2D electrophoresis, SDS-PAGE/mass spectrometry detection).

Method 1: Colorimetric Detection—CBB Staining and Silver Staining

■ SDS-PAGE+CBB Staining

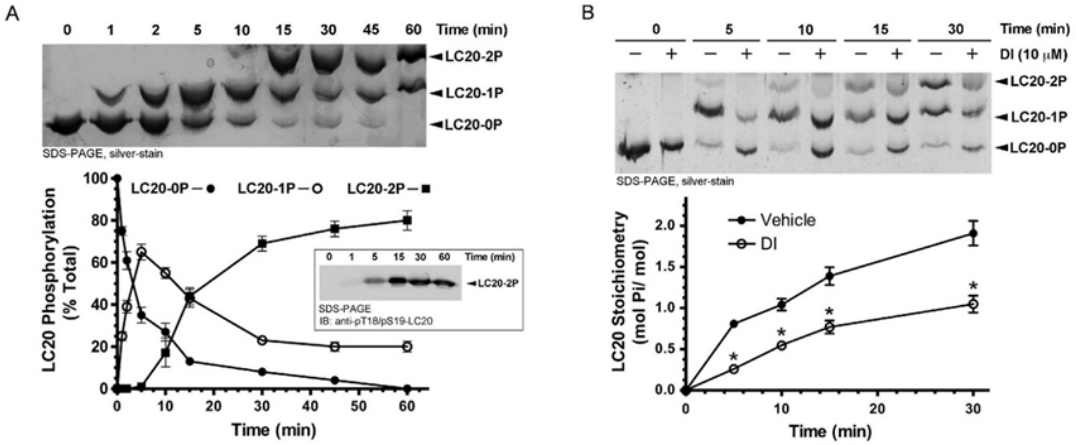


TT2 inhibits DGK7 by decreasing its phosphorylation level . Sample preparation for quantitative analysis of invitro DGK activity of DGK7 purified from Rosetta E. coli cells. Proteins were separated on regular or phos-tag SDS-PAGE gels and stained with Coomassie brilliant blue (CBB). *Cell* 2025;188(24):7378–7396.

Experimental conditions for Phosbind SDS-PAGE:

Sample	The target protein, DGK7, was expressed in Escherichia coli and purified for in vitro kinase assays.
Phosbind SDS-PAGE Separating Gel Composition	6% (w/v) gel, containing 100 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO)
In Vitro Kinase Reaction Mixture (100 μL)	40 mM Tris-HCl pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 50 μM DAG; incubated at 30°C in the dark for 60 min

■ SDS-PAGE + Silver Staining



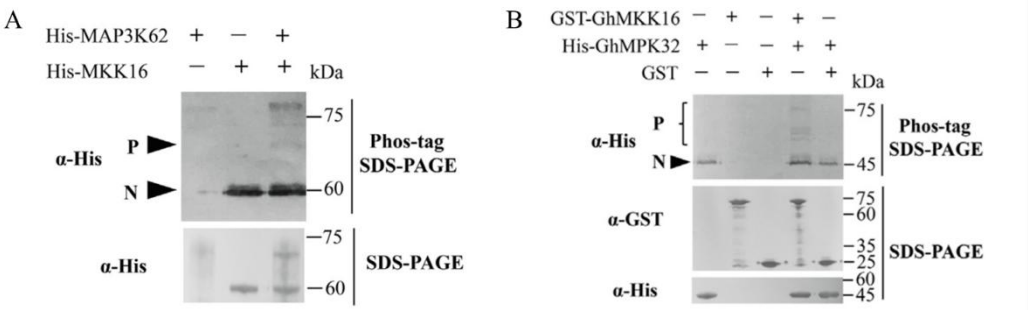
The DI compound attenuates the ROCKII-dependent phosphorylation of LC20 in vitro. (**A**) LC20 phosphorylation by ROCKII was analyzed by Phos-tag SDS-PAGE with detection of unphosphorylated (LC20-0P), mono (LC20-1P)- and di (LC20-2P)-phosphorylated forms on a silver-stained SDS-PAGE gel. The various LC20 bands were quantified from the silver-stained gel by scanning densitometry and expressed as a percentage of the total LC20 signal. A representative silver-stained gel is shown above cumulative quantitative data. Inset: a representative western blot for LC20 phosphorylation by ROCKII confirmed diphosphorylation at T18 and S19. (**B**) A representative silver-stained SDS-PAGE gel and cumulative quantitative data are provided for the time course of LC20 phosphorylation by ROCKII in the presence of vehicle (DMSO) or DI (5 μM). LC20 phosphorylation stoichiometry is expressed as mol P_i/mol LC20. *Sci Rep*. 2016 Aug 30:6:32118.

Experimental conditions for Phosbind SDS-PAGE (in vitro kinase + silver staining):

Sample	In vitro kinase reaction system, with LC20 protein as the target protein
Phosbind SDS-PAGE Separating Gel Composition	9.7% (w/v) gel, including 60 μM Mn^{2+} and 30 μM Phosbind acrylamide
In Vitro Kinase Reaction Mixture (50 μL)	25 mM HEPES buffer (pH 7.4), 2 mM $MgCl_2$, 200 μM ATP (containing 1 μCi [γ-^{32}P]-ATP), ROCKII (4 μg/mL), and 100 μM LC20 peptide; reaction at 25°C

Method 2: Chemiluminescent Detection (ECL Detection)

■ In Vitro Kinase Reaction

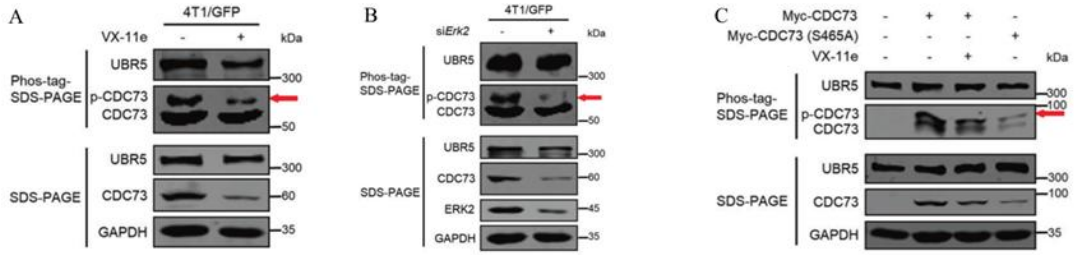


(A) GhMAP3K62 interacts with GhMKK16 and involved in improving drought tolerance in cotton. The phosphorylation of His-GhMKK16 by His-GhMAP3K62, monitored by western blot on Phos-tag gel. **(B) GhMKK16 role in drought response depends on GhMPK32.** The phosphorylation of His-GhMPK32 by GST-GhMKK16, monitored by western blot on Phos-tag gel. *J Adv Res*. 2022 Nov 19;S2090-1232(22)00249-1.

Experimental conditions for Phosbind SDS-PAGE:

Sample	Purified proteins from E. coli expression for in vitro kinase assay; target proteins are His-GhMKK16 and GhMPK32
Phosbind SDS-PAGE Separating Gel Composition	12% (w/v) gel, containing 100 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO)
In Vitro Kinase Reaction System (30 μL).	Each protein (2 μg), 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM DTT, 1 mM ATP; reaction at 30°C for 45 min

■ Cell-Based Assay (eukaryotic cells, including large MW ~300 kDa)

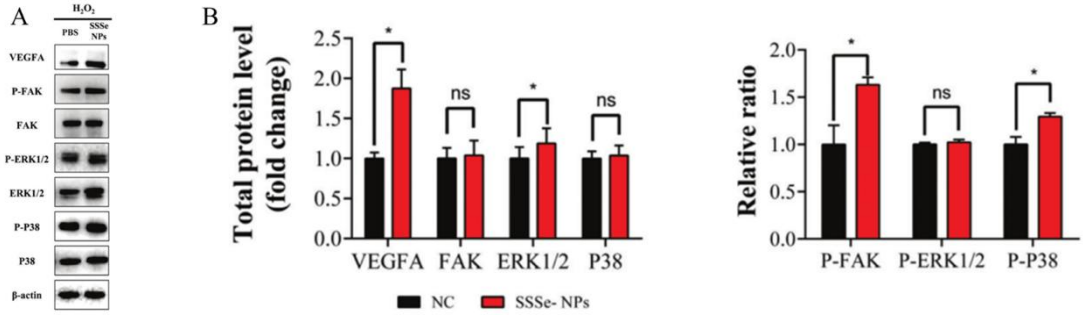


Degradation of non-phosphorylated CDC73 at Ser465 by UBR5-mediated UPS. The phosphorylation levels of UBR5 and CDC73 were analyzed via the Phos-tag SDS-PAGE in 4T1 cells treated with vehicle or VX-11e (10 μM, 24 h) (**A**) and the control and *Erk2* silenced 4T1 cells (**B**). The phosphorylation was detected in HEK293T cells treated with vehicle or VX-11e (10 μM, 24 h) (**C**) . *Cell Death Dis*. 2022 May 12;13(5):451.

Experimental conditions for Phosbind SDS-PAGE:

Sample	4T1 cell lysate; target proteins are CDC73 and UBR5 (high-molecular-weight proteins)
Phosbind SDS-PAGE Separating Gel Composition	· CDC73 protein — 6% (w/v) gel, containing 100 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO) · UBR5 protein — 3% (w/v) gel (supplemented with 0.5% agarose), containing 100 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO)

■ Cell-Based Assay (eukaryotic, including multi-band protein forms)

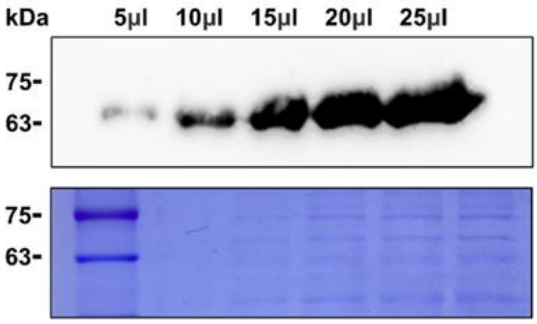


SSSe NPs administration improves angiogenesis in MI hearts. Representative Western blot showing VEGF A, p-FAK, FAK, p-ERK1/2, ERK1/2, p-P38, and P38 expression in HUVECs treated with PBS or SSSe NPs under H_2O_2 injury conditions. Phos-Tag SDS-PAGE was used to determine the phosphorylation of FAK, ERK1/2, and P38. *Adv (Weinh)*. 2023 Feb.

Experimental conditions for Phosbind SDS-PAGE:：

Sample	Human umbilical vein endothelial cell (HUVEC) lysate; target proteins are multi-band proteins (Erk, p38, VEGFA, FAK)
Phosbind SDS-PAGE Separating Gel Composition	Classic Phosbind acrylamide: $MnCl_2$ = 1: 2 molar ratio (F4002, APExBIO)

■ Cell-Based Assay (prokaryotic, purified proteins from culture supernatant)

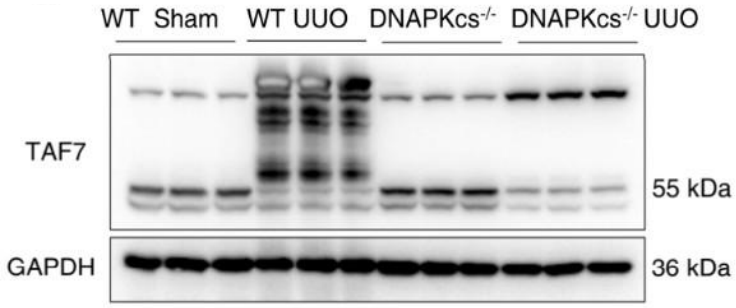


Conserved phosphopeptide binding residues of TagH are required for the expression of HlyA and Hcp in culture supernatant. TagH phosphorylation analysis using Phos-tag SDS-PAGE. *Gut Microbes*. 2022 Jan-Dec;14(1):2055440. IF: 9.434

Experimental conditions for Phosbind SDS-PAGE:

Sample	Purified Tag protein from Vibrio cholerae culture supernatant
Phosbind SDS-PAGE Separating Gel Composition	10% (w/v) gel, containing 40 μM Mn^{2+} and 20 μM Phosbind acrylamide (F4002, APExBIO)
Electrophoretic Conditions	Constant current 25–30 mA/glue, maximum voltage 120 V

■ In Vivo Experiments (mouse animal tissue)

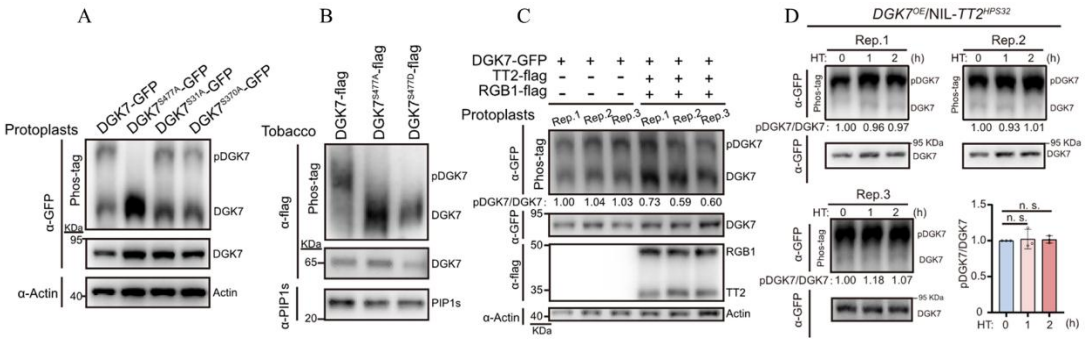


DNA-PK-mediated phosphorylation of TAF7 aggravates TGF-β1-induced tubular epithelial cell dedifferentiation and fibroblast activation. The phosphorylation of TAF7 in UUO mice was confirmed by Phos-tag SDS-PAGE. *Nat Commun*. 2023 Mar 11;14(1):1334. IF: 17.694

Experimental conditions for Phosbind SDS-PAGE:

Sample	Unilateral ureteral obstruction (UUO) mouse renal fibrosis model; tissue homogenate; target protein is TAF7
Phosbind SDS-PAGE Separating Gel Composition	8% (w/v) gel, containing 200 μM Mn^{2+} and 100 μM Phosbind acrylamide (F4002, APExBIO)

■ In Vivo and In Vitro Experiments (plant, mutation site)

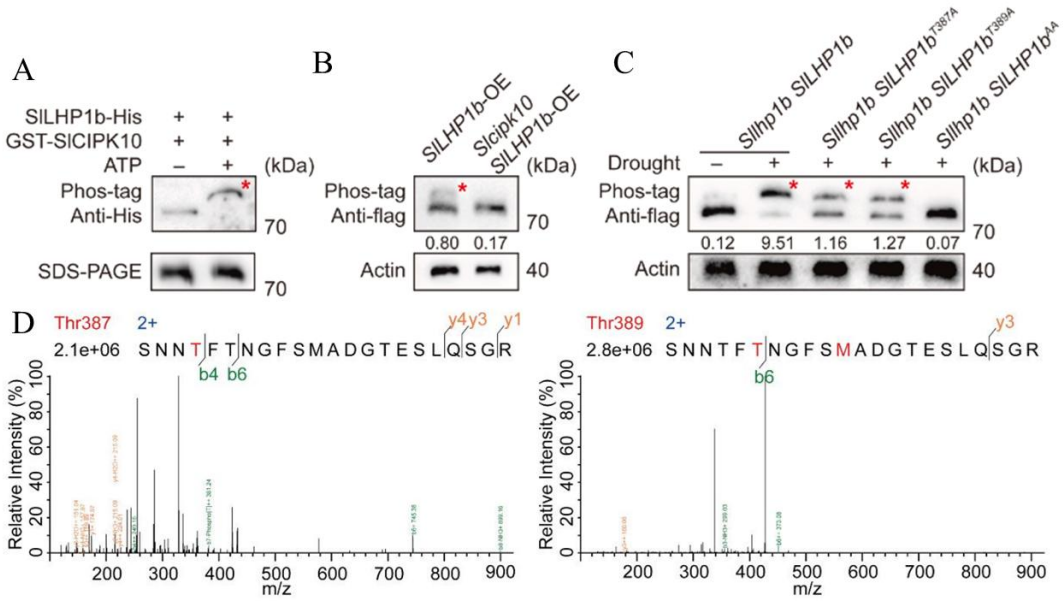


TT2 inhibits DGK7 by decreasing its phosphorylation level. (A-B) The S-A substitution at Ser 477 abolished the phosphorylation of DGK7, whereas Ser 31 and Ser 370 did not. (C) TT2 suppressed the phosphorylation of DGK7 in protoplasts. The ratio (pDGK7 to DGK7) indicated the intensity of phosphorylated DGK7 divided by non-phosphorylated DGK7, n = 3 biological replicates. (D) DGK7 phosphorylation levels under HT. *Cell*. 2025 Dec 24;188(26):7378-7396.e23.

Experimental conditions for Phosbind SDS-PAGE:

Sample	Rice protoplast lysate and whole rice seedling total protein lysate after grinding (post-IP)
Phosbind SDS-PAGE Separating Gel Composition	6% (w/v) gel, containing 100 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO)

■ SDS-PAGE followed by MS



SlCPK10-mediated SlLHP1b phosphorylation enhances protein stability and activity. **(A)** SlCPK10 phosphorylates SlLHP1b *in vitro*. The assay was conducted in the presence (+) or absence (−) of ATP. **(B)** SlCPK10 phosphorylates SlLHP1b in tomato. Total protein was extracted from the stamens of SlLHP1b-OE and Slcipk10 SlLHP1b-OE lines under drought conditions. **(C)** SlLHP1b is phosphorylated on Thr387 and Thr389 in stamens under drought conditions. Total protein was extracted from the stamens of the indicated genotypes. **(D)** Liquid chromatography–tandem mass spectrometry (LC–MS/MS) revealing the phosphorylation sites of SlLHP1b. Ge S, et al. *Dev Cell*. 2025;60(20):2730-2743.e9.

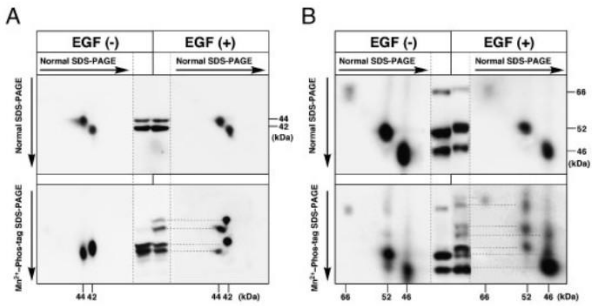
Experimental conditions for Phosbind SDS-PAGE: (Plant in Vitro Kinase Experiment and In Vivo Experimental Samples):

Sample	Plant (tomato) in vitro kinase experimental samples and in vivo experimental tissue samples
Phosbind SDS-PAGE Separating Gel Composition	8% (w/v) gel, containing 50 μM Mn^{2+} and 50 μM Phosbind acrylamide (F4002, APExBIO) vs. Control group (conventional SDS-PAGE): 10% (w/v) gel
In Vitro Kinase Reaction System (30 μL).	1.5 μg recombinant GST-SlCIPK10, 1.5 μg SlLHP1b-His, 20 mM Tris-HCl, 2.5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.5 mM $CaCl_2$, 2 mM DTT, 1 mM ATP; reaction at 37°C for 1 h. Terminated by adding protein loading buffer and boiling.
LC–MS/MS for Phosphating Site Identification	In vitro kinase reaction products were separated by Phosbind SDS-PAGE. Gel bands showing retarded migration (phosphorylated SlLHP1b-His) were excised and subjected to in-gel tryptic digestion (10 ng/μL trypsin, overnight at 25°C). Resulting peptides were analyzed by LC-MS/MS.

Method 3: Two-Dimensional Electrophoresis

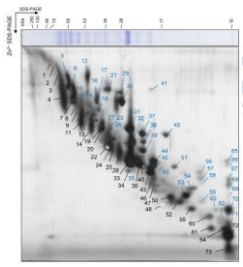
■ 2D Electrophoresis (1D SDS-PAGE; 2D Mn^{2+}/Zn^{2+}-phosbind SDS-PAGE)

Mn^{2+}-Phos-tag



2-D mobility shift detection of the phosphoisotypes of MAPK and Shc by coupling SDS-PAGE and Mn^{2+}–Phos-tag SDS-PAGE followed by immunoblotting. (A) Separation of the phosphoisotypes in each MAPK isoform (45 and 42 kDa). (B) Separation of the phosphoisotypes in each Shc isoform (66, 52, and 46 kDa). The protein samples (20 mg protein/lane) from A431 cells before (-) and after (+) EGF stimulation (50 ng/mL, 5 min) were applied to first-dimensional normal SDS -PAGE (10% w/v polyacrylamide for MAPK analysis, 7.5% w/v polyacrylamide for Shc analysis). *Electrophoresis*. 2009 Feb;30(3):550-9.

Zn^{2+}-Phos-tag



Identification of protein spots detected on 2D Phos -tag SDS-PAGE by LC–MS/MS. The protein spots on the 2D Phos-tag SDS-PAGE in this figure were serially numbered. Blue and black numbers, respectively, denote protein spots shifted and non-shifted upward, compared with the gel without Phos-tag. The Phos-tag gel consisted of a separating gel [10% (w/v) acrylamide, 350 mM Bis–Tris (pH 6.8), 25 μM Phos-tag acrylamide, 100 μM ZnCl2]. *Photosynth Res.* 2021 Jan;147(1):107-124.

Experiment 1 (Left):	1D: SDS-PAGE; 2D: Mn^{2+}-Phosbind SDS-PAGE
Sample	A431 cell lysate (± EGF stimulation)
Phosbind SDS-PAGE Separating Gel Composition	1D: ● A-10% (w/v) gel ● B-7.5% (w/v) gel 2D: ● A-10% (w/v) gel, containing 25 μM Mn^{2+} and 50 μM Phosbind acrylamide. ● B-7.5% (w/v) gel, containing 25 μM Mn^{2+} and 50 μM Phosbind acrylamide.
Detection methods	ECL
Experiment 2 (Right):	1D: SDS-PAGE; 2D: Zn^{2+}-Phosbind SDS-PAGE
Sample	Arabidopsis thaliana thylakoid membrane proteins
Phosbind SDS-PAGE Separating Gel Composition	1D: 10% (w/v) gel 2D: 10% (w/v) gel, containing 25 μM Zn^{2+} and 100 μM Phosbind acrylamide.
Detection methods	Silver staining

Mn^{2+}/Zn^{2+}-Phosbind SDS-PAGE: Differences and Selection Guide:

Mn^{2+}-Phosbind SDS-PAGE: Compatible with standard Laemmli alkaline Tris-Gly system (pH ≈ 9). Under alkaline conditions, Mn^{2+} forms a moderately reversible complex with phosphate groups. Differences in steric hindrance at phosphorylation sites produce stable electrophoretic mobility shifts, enabling discrimination of protein

isoforms with the same phosphorylation number but different phosphorylation sites. Suitable for samples requiring separation of same-phosphorylation-number/different-site isoforms, compatible with phosphorylation site mapping by mass spectrometry.

Zn^{2+}-Phosbind SDS-PAGE: Compatible with neutral Bis-Tris system (pH 6.8). Zn^{2+} exhibits stronger affinity for phosphate group capture. The neutral environment prevents membrane protein aggregation under alkaline conditions, yielding clean, sharp bands with lower background. However, the excessively strong metal-phosphate chelation in this system completely masks the subtle mobility differences caused by different phosphorylation sites, making it impossible to distinguish protein isoforms with the same phosphorylation number but different sites. Suitable only for bulk screening of phosphorylation presence/absence.

SDS-PAGE/MS Identification Workflow (Reference)

This protocol is applicable for in-gel digestion of protein bands or spots stained with Coomassie Brilliant Blue (CBB) or silver, followed by analysis via MALDI-MS or LC-MS/MS.

Protocol

1. Reagent Preparation:

- 100 mM NH_4HCO_3: 0.790 g dissolved in 100 mL ultrapure water. Freshly prepared daily, discarded after use.
- 10 mM DTT:1.5 mg DTT + 1 mL 100 mM NH_4HCO_3. Prepare immediately before use.
- 55 mM iodoacetamide (IAA): 10.2 mg IAA + 1 mL 100 mM NH_4HCO_3. Prepare and use immediately, store away from light.
- Trypsin solution: 20 μg Trypsin + 1.5 mL 10 mM NH_4HCO_3 / 10% ACN (final concentration 13 ng/μL). Ready to use as needed, discard unused parts.
- Peptide extraction buffer: 5% FA: ACN = 1:2 (i.e., 5% formic acid/acetonitrile mixture)
- 0.1% TFA complex solution: 0.1% TFA compound solution

2. Gel Band (Spot) Pretreatment

After electrophoresis, follow the specific instructions for Coomassie Brilliant Blue staining or silver staining. Take Coomassie Brilliant Blue dyeing as an example:

a. Fixation: Soak the gel in a fixative (50% (v/v) methanol, 2% (v/v) phosphoric acid), gently shake for at least 30 minutes.

b. Washing: Pour out the stationary solution and wash with deionized water three times, each time for about 10 minutes.

c. Incubation: Soak in incubation solution (34% methanol, 17% ammonium sulfate, 2% phosphoric acid), gently shake for 30 minutes

d. CBB staining: Discard the incubation solution, add staining solution (34% methanol, 17% ammonium sulfate, 2% phosphoric acid, 0.066% colloidal Coomassie Brilliant Blue G-250), and stain overnight on a shaker.

e. Destaining: Wash several times with deionized water to remove excess dye.

f. Visualization: Scan and record gel images.

*Note: 1. It is not recommended to directly perform silver staining on gel samples with unknown protein components in experiments. Coomassie Brilliant Blue staining should be used first. If needed, silver staining can be performed directly without decolorization. 2. Intra-gel enzymatic hydrolysis is

suitable for most silver staining schemes, but the use of reagents that covalently modify proteins such as glutaraldehyde and strong oxidants like chromates/permanganates is strictly prohibited.

3. Excise Protein Bands (Spots) (5 minutes)

a. Rinse the entire one- or two-dimensional gel block with water for several hours, place the plastic tray containing the gel on the viewing lamp, and use a clean scalpel to cut off the target band (protein spot).

*Note: Wear powder-free gloves at all times and use clean utensils to avoid keratin contamination.

b. Cut the cut strips (egg white dots) into small cubes about 1 mm.

*Note: Note that if the gel block is too small, it can easily clog the pipette tip.

c. Transfer the gel pieces into centrifuge tubes and perform brief centrifugal settling using a benchtop microcentrifuge.

*Note: Phosind SDS-PAGE gel is used for mass spectrometry analysis without special steps such as EDTA treatment.

4. Removal of Gel Components (Mn2+, etc.) (Optional)

a. Add 100 μL of Solution A (10 mM NH_4HCO_3, 10 mM EDTA) and incubate at room temperature for 10 min. Discard Solution A.

b. Add 100 μL of Solution B (5 mM NH_4HCO_3, 50% ethanol, 5 mM EDTA) and incubate at room temperature for 10 min. Discard Solution B. Repeat this cycle twice more (3 cycles in total) to thoroughly remove Mn^{2+}.

c. Remove the final liquid completely.

5. In-Gel Reduction, Alkylation, and Destaining (30–60 min)

■ In-gel reduction, alkylation and destaining of proteins

a. Add 500 μL of pure acetonitrile (ACN) to the above centrifuge tube, incubate the tube for 10 minutes until the plastic block wrinkles (becomes opaque and adheres to each other).

b. Centrifugal precipitation of the rubber block, completely discarding all liquid.

c. Reduction: Add 30-50 μL of 10 mM DTT to completely cover the rubber block, then incubate in an air incubator at 56°C for 30 minutes.

d. Cool the centrifuge tube to room temperature (about 22°C), add 500 μL acetonitrile, incubate for 10 minutes, then completely remove all liquid.

e. Alkylation: Add 30-50 μL 55 mM iodoacetamide solution (solution volume sufficient to submerge the plastic block), incubate at room temperature in the dark for 20 minutes.

f. Add another 500 μL acetonitrile to dehydrate and wrinkle the block, then remove

all liquid.

- Destain gel pieces excised from Coomassie-stained gels

a. Decolorization: Add about 100 μL of 100 mM decolorization solution (NH_4HCO_3 /acetonitrile, v/v=1:1), incubate with intermittent vortex oscillation for 30 minutes according to the staining depth. Decide whether to change the liquid as needed.

b. Dehydration: Add 500 μL pure acetonitrile, incubate with intermittent vortex oscillation at room temperature until the glue blocks turn white and wrinkle, then discard the acetonitrile. At this point, most Coomassie brilliant blue dye should have been removed, and there is no need to completely decolorize the glue block.

*Note: At this point, the sample can be directly hydrolyzed in gel or stored at -20°C for several weeks.

c. Reduction and alkylation (optional): For specific operations, please refer to 5.b-f. Choose as needed.

*Note: Residual small amounts of CBB staining do not affect mass spectrometry identification.

6. Saturate gel pieces with trypsin (120 minutes)

a. Add enough trypsin solution to cover the dry glue block (usually 50 μL or more, with the exact amount adjusted according to the volume of the gel matrix), then place on ice or at 4°C.

b. After about 30 minutes, check whether the buffer solution is fully absorbed. If necessary, add trypsin solution, making sure the glue block is fully submerged in the solution.

d. Continue to leave the block for 90 minutes to allow trypsin to fully penetrate it, then add 10~20 mL ammonium bicarbonate buffer (100 mM) to soak the block, ensuring the block remains moist during digestion.

*Note: Even if the block no longer absorbs liquid after 30 minutes, extending the swell to 120 minutes can still significantly increase peptide yield, as the diffusion of enzymes in the polyacrylamide matrix is a rate-limiting step.

7. Digestion (30 minutes ~ overnight)

Place the centrifuge tube containing the gel particles into an air circulation incubator, then selectively treat the condition:

- Overnight mode (37±1°C, 12-16 hours or overnight, recommended): If the experiment needs to be conducted at the maximum sensitivity of the instrument, peptide recovery should be maximized.
- Fast mode (55±1°C, 30 minutes): Typically used for rapid identification of

Coomassie brilliant blue stainable protein spots (bands) by matrix-assisted laser desorption ionization mass spectrometry fingerprinting, quickly achieving qualified hydrolysis efficiency, but only about 75% of overnight hydrolysis efficiency.

If the proteolysate product is further tested using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, perform MALDI sampling (Step 7); If no further testing is needed, you can jump directly to the peptide extraction step (Step 8).

*Note: Avoid temperature differences between the bottom of the pipe and the cap, and prevent water vapor from condensing on the inner wall of the cap, which could cause the gel particles to dehydrate and dry out prematurely.

8. MALDI Sampling (optional)

Only when subsequent MALDI-TOF MS peptide mass fingerprinting (PMF) identification is performed:

Centrifuge tubes are cooled to room temperature, and centrifuge blocks are used in microcentrifuges to directly draw 1–1.5 μL of supernatant from the enzymatic hydrolysis system, without the need for additional extraction operations. Remaining samples can be reserved for subsequent LC-MS/MS analysis, and can be stored for several months at -20°C.

9. Extract Peptide Digestion Products (15 minutes)

a. Add 100 μL of peptide extraction buffer (5% formic acid : acetonitrile = 1:2) to each centrifuge tube and incubate in a shaker at 37°C for 15 minutes. If the gel matrix sample is too large or too small, adjust the amount of extraction buffer so that the volume ratio of hydrolysate to extraction buffer is about 1:2.

b. The supernatant is drawn using a pipette gun equipped with a fine gel loading tip, transferred to a PCR tube, and dried using a vacuum centrifuge concentrator. The dried extract can be stably stored at -20°C for several months.

*Note: 1. When drawing supernatant, use a pipette gun equipped with a fine gel loading tip to avoid clogging the injector needle or nanofluidic liquid chromatography-tandem mass spectrometry column. 2. Keep the glue block and do not discard it. If enzymatic hydrolysis fails, it can be reprocessed. 3. For particularly large or very small rubber blocks, adjust the amount of extract according to the ratio of enzymatic hydrolysate volume to extract ≈ 1:2.

10. Redissolve Tryptic Peptides for Further Analysis

a. Redissolving peptides: Add 10-20 μL of 0.1% TFA complex solution to the centrifuge tube, vortex and mix well.

b. (Optional) If phosphopeptide enrichment is required, TiO_2 chromatography can be performed at this time.

c. Auxiliary extraction: vortex oscillation and/or ultrasonic water bath treatment for 2–5 minutes, promoting full release of peptides from gel particles or precipitates into solution through mechanical disturbance and cavitation effects.

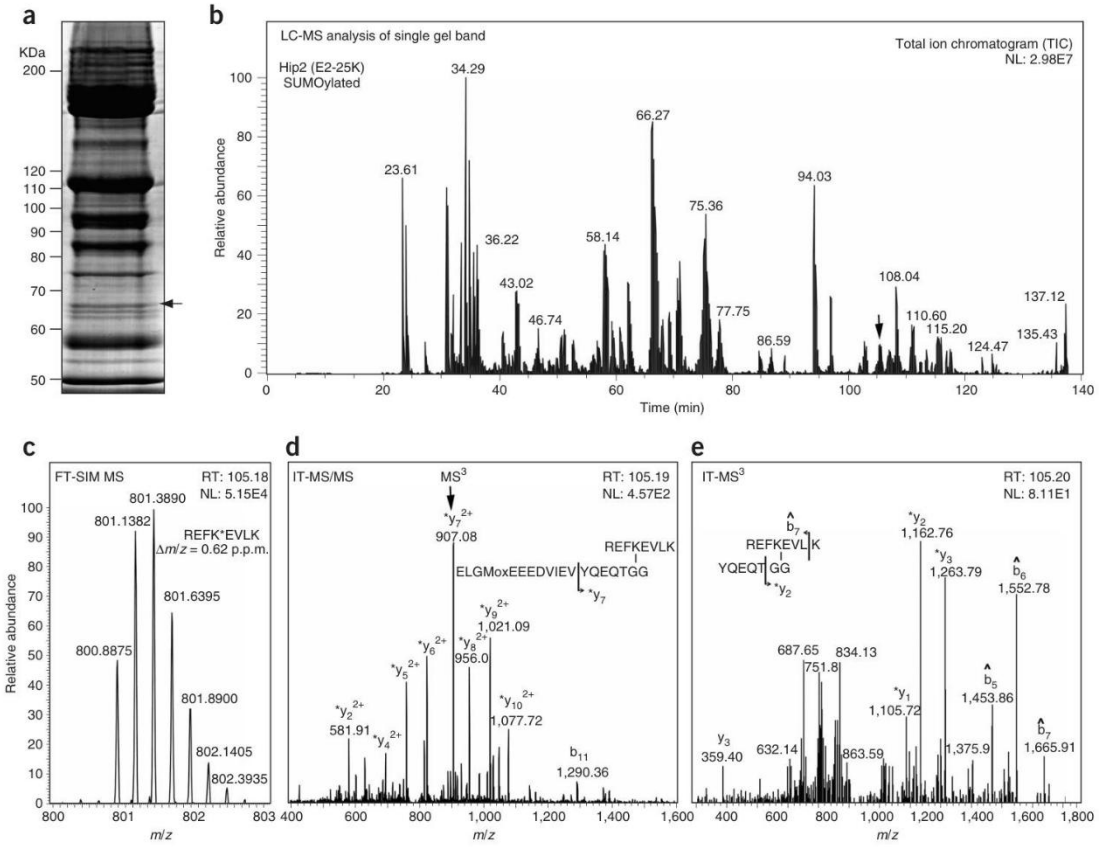
d. Centrifugal separation: Centrifuge at 6.7g (10,000 rpm) for 15 minutes using a benchtop centrifuge.

e. Sampling for later use: Draw an appropriate amount of supernatant for subsequent testing (such as LC-MS/MS analysis). After vacuum centrifugation and drying, store the remaining sample at -20°C for later use.

*Note: For further purification, desalting can be done using STAGE tip or ZipTip before machine installation (see Rappsilber et al., Anal Chem 2003).

11. Result Analysis

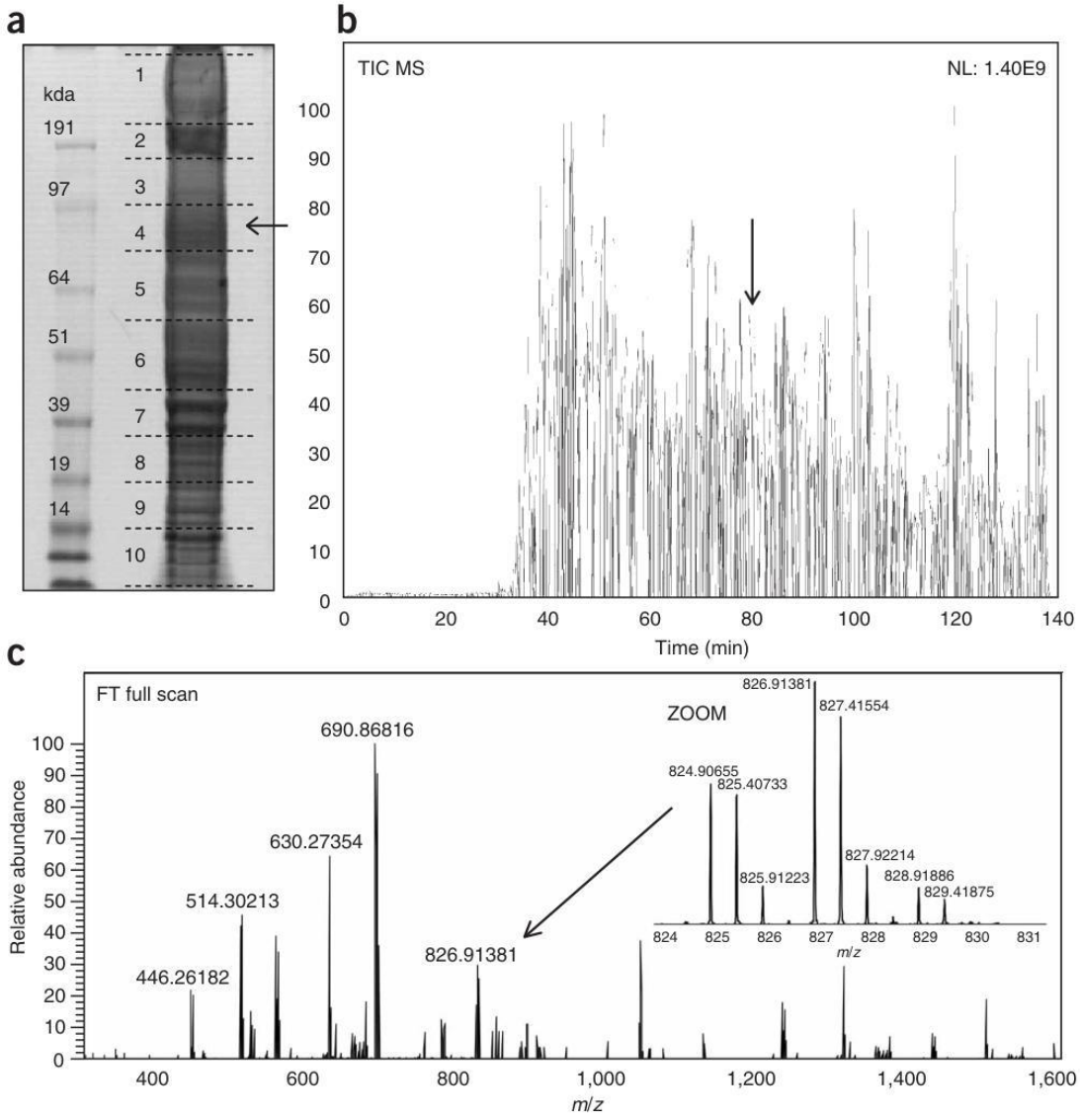
■ Single-strip post-translational modification (SUMO-ization) GeLC-MS analysis



(a) Gel electrophoresis: GST-SUMO1 pull-down samples were stained with CBB, with arrow-marked bands taken and detected by LTQ-FT liquid chromatography-mass

spectrometry (LC-MS) tandem mass spectrometry, identifying a total of 20 peptides, confirming that the band proteins were Huntingtin-acting protein Hip2; (b) Total ion flow map: Displays the total ion efflux peak signal of Hip2-related peptides during chromatographic elution; (c-e) Multi-level mass spectrometry (primary mass spectrometry, secondary MS/MS, tertiary MS^3 spectra): Precisely lock the SUMO modification site at lysine (K14) at position 14 of the Hip2 protein.

■ Whole-cell complex proteome stratified analysis by GeLC-MS



(a) Total protein lysis solution from HeLa cells was extracted, separated by one-dimensional gel electrophoresis, and cut into 10 gel strips. Each strip was enzymatically hydrolyzed according to the experimental protocol; (b) Select the diagram (a) Use the middle arrow to mark the rubber strip and plot the total ion flow map; Due to the large number of eluted peptides, individual chromatographic peaks cannot be distinguished; (c) The arrows in Figure (b) indicate the full-scan mass

spectra corresponding to the elution time points, which include at least 30 groups of co-eluted stable isotope-labeled amino acid (SILAC) trimodal peptides.

Reference:

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