Proteomics Sample Preparation Kit for Cultured Cell (#FMR-001)

Proteomics Sample Preparation Kit for Cultured Cell enables to complete sample preparation for proteomics analysis by LC-MS/MS, especially for MRM analysis with mTRAQ® reagent (AB Sciex Pte. Ltd.). This kit contains the necessary reagents for cell lysis, protease digestion, and alkylation.

For research use only. Not for use in diagnostic procedures.

Avoid freeze-thaw cycles.

Components (for 10 preps)

- □ ① Tris HCl（700 µl）
- □ ② SDS（250 µl）
- □ ③ Urea（4.24 g）
- □ ④ Digestion Buffer（0.4 ml）
- □ ⑤ Protease A（1 vial）
- □ ⑥ Protease B（2 vial）
- □ ⑦ Reagent A（10 vials）
- □ ⑧ Reagent B（10 vials）
- □ ⑨ Reagent C（1 vial）
- □ ⑩ Storage Tubes（20 tubes）

Reagent Preparation

Cell Lysis Buffer: Add 5.7 ml of ultrapure water to the Urea bottle (Component ③) just prior to use, and mix thoroughly. Add 700 µl of Tris HCl (Component ①) and then add 250 µl of SDS (Component ②). Mix throughly.

Protease A: Add 12 µl of ultrapure water to the vial. Store unused solution at –20 ºC for 1 month.

Protease B: Add 24 µl of ultrapure water to each vial. Store unused solution at –20 ºC for 1 month.

Reagent A, B: Add 100 µl of ultrapure water just prior to use.

Reagent C: Add 1 ml of ultrapure water and dispense 100 µl to each storage tube. The solution is stable for 1 month at –20 ºC.

Storage

①～④, ⑦～⑨**:** 4 ºC, ⑤⑥**:** –20 ºC, ⑩**:** RT

Protocols

Step A. Cell Lysis

[Materials required but not provided]

- □ Phosphate buffered saline (PBS)
- □ Trypsin soluton
- □ DMEM medium with 10%FBS
- □ BSA standard solution (20 mg/ml)
- □ BCA assay kit
- □ Coulter counter
- □ Probe type sonicator (Frequency 20 kHz, Power Rating 85 W, Probe Size Φ2 mm, or equivalent

[General Protocol]

Case A. Cell lysis from detached cells

(1) Wash cultured cells of 10 cm dish with 10 ml of PBS.
(2) Add 1 to 2 ml of Trypsin solution, then incubate at 37 ºC for 5 min.
(3) Add 8 ml of DMEM medium with 10%FBS to the culture dish.
(4) Transfer the detached cell suspension to 15 ml tube.
(5) Centrifuge and discard supernatant.
(6) Wash the cell pellet with 10ml of PBS.
(7) Centrifuge and discard supernatant.
(8) Repeat step 6 and 7 twice.
(9) Resuspend the cell pellet in PBS (2 to 4 ml).

NOTE: You should optimise the cell detachment steps above, depending on the cells you use.

(10) Count the cell number of 75 µl cell suspension.
(11) Transfer approx. 2×10^6 cells to 1.5 ml tube, centrifuge and discard supernatant.
(12) Add 200 µl of **Cell Lysis Buffer**.
(13) Sonicate 30 sec. for twice.
(14) Add 200 µl of ultrapure water.
(15) Sonicate 30 sec. for twice.
(16) If the solution is still viscous, repeat sonication.

Case B. Direct lysis from Cell Culture Dish

(1) Wash cell culture of 10 cm dish with 10 ml of PBS for 3 times.

NOTE: Use cell culture dish of 3×10^6 to 1×10^7 cells.

(2) Add 500 µl of **Cell Lysis Buffer** and spread whole area of culture dish.
(3) Add 500 µl of ultrapure water and mix well.
(4) Use cell scraper and gather the cells to the edge of the dish.
(5) Sonicate 30 sec. for 3 times or untill the solution become low viscous.
(6) Transfer the lysate to 1.5 ml tube. If the solution is still viscous, repeat sonication.

BCA assay

a. BSA standards preparation: 10-fold dilution of BSA standard solution (20 mg/ml) with the 2-fold diluted **Cell Lysis Buffer.** Prepare BSA

standards of 0, 0.5, 1, 1.5, 2 mg/ml using 2-fold diluted **Cell Lysis Buffer.**

b. Add 10 µl of BSA Standards or sample per well.
c. Dispense 200 µl of BCA solution per well.
d. Incubate at 37 ºC for 30 min.
e. Read at 570 nm. Calcurate protein concentrations.

Step B. Peptide Sample Preparation

[Materials required but not provided]

- ☐ 100% Methanol
- ☐ 80% Methanol
- ☐ Chloroform
- ☐ BSA standard solution (20 mg/ml)
- ☐ BCA assay kit
- ☐ mTRAQ® Reagent (AB SCIEX, #4374771)
- ☐ Isopropanol

[Protocol]

Methanol/Chloroform Precipitation

(1) Transfer 100 to 200 µg protein into **Storage Tube.** Fill with 2-fold diluted **Cell Lysis Buffer** to 200 µl. You can store the solution at –20 ºC

NOTE: Be careful not to exceed 200 µl. If the protein concentration is low, divide the cell lysate solution into 2 **Storage Tubes** and conduct following step (2) to (17) for each tube, add 14 µl of **Digestion Buffer** to the each tube and then combine the solution.

(2) Add 600 µl of 100% Methanol and vortex.
(3) Add 200 µl of Chloroform and vortex.
(4) Add 400 µl of ultrapure water and vortex.

NOTE: The solution become clouded after Step (4).

(5) Incubate on ice for 30 min.
(6) Centrifuge 13,000×g for 5 min.
(7) Discard the supernatant.
(8) Add 600 µl of cold 100% Methanol and vortex.
(9) Centrifuge 2,100×g for 5 min.

NOTE: Use swing bucket rotor centrifuge for this step.

(10) Centrifuge 13,000×g for 2 min.
(11) Discard supernatant remaining 100 µl aliquot.
(12) Flush centrifuge and disgard the remaining liquid.
(13) Add 1.5 ml of 80% Methanol and vortex.
(14) Centrifuge 2,100×g for 5 min.

NOTE: Use swing bucket rotor centrifuge for this step.

(15) Centrifuge 13,000×g for 2 min.
(16) Discard supernatant remaining 100 µl aliquot.
(17) Flush centrifuge and disgard the remaining liquid.
(18) Add 28 µl of **Digestion Buffer**.
(19) Incubate at 65 ºC for 15 min.
(20) Leave the solution to ambient temperature.
(21) Add 28 µl of ultrapure water.
(22) Conduct following BCA assay.

a. BSA standards preparation: 2-fold dilution of BSA standard solution (20 mg/ml) with 2-fold diluted **Digestion Buffer**. Prepare BSA standards of 0, 1.25, 2.5, 5, 10 mg/ml using 2-fold diluted **Digestion Buffer**.
b. Add 2 µl of BSA Standards or sample per well.

NOTE: Conduct BCA assay in triplicate.

c. Dispense 200 µl of BCA solution per well.
d. Incubate at 37 ºC for 30 min.
e. Read at 570 nm. Calcurate protein concentrations.

Protease digestion

(1) Add 50 µl of ultrapure water to the sample.
(2) Add 1 µl of **Protease A**.
(3) Incubate at 37 ºC for 3 hours.
(4) Add 100 µl of ultrapure water and mix by tapping.
(5) Add 1 µl of **Protease B**.
(6) Incubate at 37 ºC for 3 hours.
(7) Add 1 µl of **Protease B**.
(8) Incubate at 37 ºC overnight.
(9) Add 2.5 µl of **Reagent A**.
(10) Incubate at 37 ºC for 30 min, then incubate at RT for a few minutes.
(11) Add 2.5 µl of **Reagent B**.
(12) Incubate at RT, dark for 30 min.
(13) Add 1 µl of **Reagent C**.
(14) Incubate at RT for 30 min.
(15) Lyophilize at –65 ºC.

Labeling with mTRAQ® Reagent

(1) Add 20 µl of ultrapure water.
(2) Mix by tapping.
(3) Add 50 µl of Isopropanol and vortex.
(4) Add 20 µl of mTRAQ Δ0 (1.0 U). Vortex and spin down.
(5) Incubate at RT for 90 min.
(6) Add 150 µl of ultrapure water. Incubate at RT for 30 min.
(7) Incubate at –80 ºC for 1 hour.
(8) Lyophilize at –65 ºC for several hours.
(9) Store at –80 ºC until analysis.
(10) Just before analysis, reconstitute with ultrapure water at 0.2 µg/µl concentration.

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