Sample clean-up: Salt	
Sample type: Cell, Tissue, Blood sample	**Method: Sep-pak**

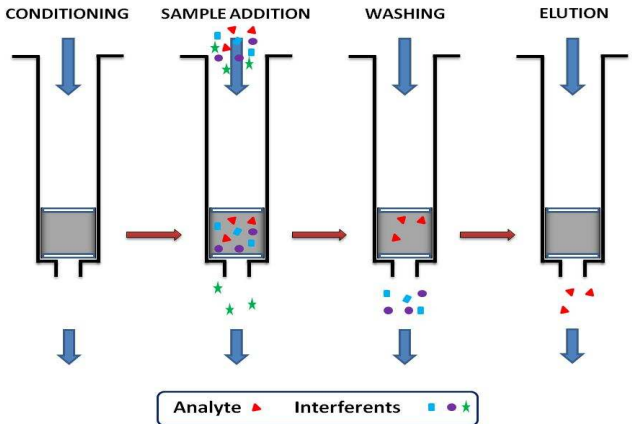
2015. 8.

K-Bio 신약개발지원센터 진종화

Removal of salts reduces streaking and improves reproducibility of 2-D gels. Use either buffer exchange (desalting) or protein precipitation (which can also help concentrate the sample if needed).

Methods to remove nucleic acids from sample:

- **Dilution** — only works if the protein concentration is high in the starting sample
- **Dialysis** — some proteins may adsorb to the dialysis membrane, however a detergent usually prevents this; often used under native condition where denaturation must be avoided
- **Protein precipitation** — the most versatile method to selectively separate proteins from contaminants consists of protein precipitation by trichloroacetic acid (TCA)/acetone followed by resolubilization in electrophoresis sample buffer
- **Buffer Exchange** — size exclusion chromatography is another effective method for removing salts, detergents, and other contaminants



1. The digested peptide mixture was applied onto an HLB Oasis cartridge for desalting
2. For each digested sample, an Oasis cartridge was washed with 2 ml 90% acetonitrile: 0.1% formic acid, equilibrated with 5 ml of 0.1% formic acid, loaded with digested sample, and washed with 3 ml 0.1% formic acid.
3. The sample was eluted using 1 volumes of 1 ml of 40 to 90% acetonitrile: 0.1% formic acid, and vacuum centrifuged to dryness.

Sample clean-up: Salt	
Sample type: Cell, Tissue, Blood sample	**Method: ZipTip method**

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K-Bio 신약개발지원센터 진종화

Desalting by ZipTip method

Solutions required preparing before starting ZipTip:

1. Acetic acid (1%)
2. Acetonitrile (100%)
3. Formic acid (0.4%)
4. 0.4% acetic acid in 50% CAN

Steps:

1. Dissolve the dried protein sample in 10 µl 1% acetic acid.
2. Activate the ZipTip by 100% ACN (10 µl) ten times.
3. Equilibrate the ZipTip by 0.4% formic acid (10 µl) ten times.
4. Adsorb the sample by pipetting 10-20 times to bind the peptides to the ZipTip.
5. Wash the ZipTip by 0.4% formic acid (10 µl) ten times.
6. Elute the peptide from the ZipTip by using 0.4% acetic acid in 50% ACN (10-20 µl)-by pipetting several times in another tube.
7. Dry the sample in speed-vac.
8. Store the sample at -20 °C before LC/MS analysis.

Sample clean-up: Salt	
Sample type: Cell, Tissue, Blood sample	**Method: Acetone precipitation**

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K-Bio 신약개발지원센터 진종화

1. Cool the required volume of acetone to -20 °C.
2. Place protein sample in acetone-compatible tube (200 µl)
3. Add 6.5 times the sample volume of cold (-20 °C) acetone (1.3 ml) to the tube (total 1.5 ml)
4. Voltex tube and incubate for 10 min at -20 °C.
5. Centrifuge (12,000 rpm, 4 °C, 10 min)
6. Decant and properly dispose of the supernatant, being careful to not dislodge the protein pellet
7. Allow the acetone to evaporate from the uncapped tube at room temperature for 10 min. Do not over-dry pellet, or it may not dissolve properly
8. Add 100 µl of labeling buffer
9. Incubate 30 min at 37 °C with shaking (for complete denature)
10. Quantitation the protein with Bradford assay

■ **Labeling buffer**

- 0.05% SDS,
- 50 mM Tris pH 8.3
- 5 mM EDTA
- 6 M Urea

Sample clean-up: Salt	
Sample type: Cell, Tissue, Blood sample	**Method: TCA precipitation**

2015. 8.

K-Bio 신약개발지원센터 진종화

1. Add TCA (trichloro acetic acid, SIGMA, 097K6156) to the sample to bring the TCA concentration to 20% (usually we can uptake 1,910 µl of depleted plasma if initially using 40 µl of plasma for depletion, so have to add TCA 382 µl)
2. Incubate on ice at least 1hr. dilute samples may be left overnight
3. Centrifuge (13,000 x g, 4 °C, 10 min)
4. Wash the pellet with a solution of ice cold acetone
5. Stand for 10 min in ice
6. Centrifuge (13,000 x g, 4 °C, 10 min)
7. Repeat 5 and 6, 2 times more
8. Add 100~200 µl of resuspend buffer
9. Incubate 30 min at 37 °C with shaking (for complete denature)
10. Quantitation the protein with Bradford assay (2 fold dilution)

■ **Resuspend buffer**

- 0.05% SDS,
- 50 mM Tris pH 8.3
- 5 mM EDTA
- 6 M Urea