MonoTip® Trypsin Pipette Tip Operation Instructions

Thank you for purchasing the MonoTip® Trypsin pipette tips.
The MonoTip® Trypsin is a filter type sample preparative tip that consists of silica monolith with a continuous through-pore skeleton with TCPK (N-p-tosyl-L-phenylalanyl chloromethyl ketone) treated trypsin (Bovine) chemically bonded. MonoTip® Trypsin pipette tip is a very useful tool for prompt protein digestion. To maintain optimum performance, read the following instructions before use.

1. Unpacking

○ Check that there are no irregularities in the external appearance of the tip, the packaging, and the buffer solutions.

2. Handling the Tips

- ○ Do not drop or bump the tips. Subjecting the tip to shocks may damage the monolith silica gel.
- ○ Do not autoclave.
- ○ The MonoTip® Trypsin pipette tip includes 0.02% aqueous sodium azide as a preservation solution. Do not dry up the tips before use.

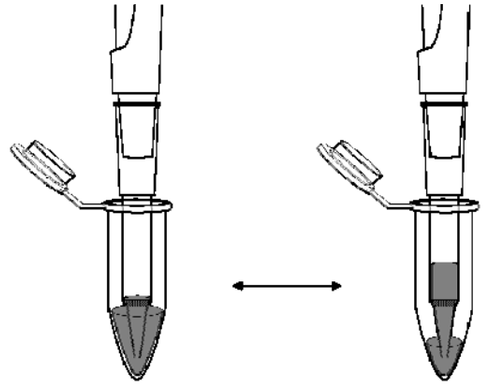
3. Storage

- ○ Store the MonoTip® Trypsin pipette tips in a refrigerator (4 – 8 ℃).

4. Care and Attention

- ○ The volume of the MonoTip® Trypsin pipette tip is 200μL. Use a suitable pipette. We recommend the Gilson Pipetman P200.
- ○ Use highly purified water and reagent grade buffers.
- ○ The MonoTip® Trypsin pipette tips are designed for single-use.
- ○ Throughout the procedure, depress and release the plunger slowly to ensure optimal solution movement through the monolith bed but do not let air reach the monolith bed. (See the picture)
  Increasing the number of drawing and ejecting samples may improve the digestion efficiency.

- ○ Use aqueous acetonitrile of concentrations less than 20%. Higher concentrations of acetonitrile may cause enzyme deactivation. Before digestion by MonoTip® Trypsin, reduction treatments, protein S-alkylations and sample desalting must be completed. Proteins can not be digested without reduction, S-alkylation and desalting.



Depress the Plunger　　Release the Plunger

Example of Reduction, S-Alkykation and Desalting

Protein Sample 1mg
| ← Protein solubilization and reduction reagent (Ex: 7M guanidine hydrochloride, 1M Tris HCl (pH 7.5),10 mM DTT=dithiothreitol)
| ← Shake (2 hours at 37℃)
N2 Blow
| ← Add iodoacetamide (2.5 times volume of the protein), cover the whole vial with aluminum foil to shield from light
| ← Shake (2 hours at room temperature)
| ← Desalting (Gel Filtration Column or MonoTip C18)
Sample after reduction, S-alkylation and desalting

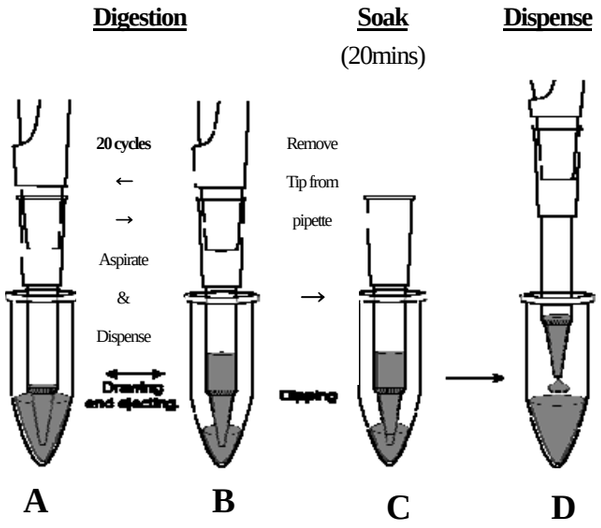
5. Example of Typical Digestion Protocol

1. Set the pipette to 200μL and attach a MonoTip® Trypsin pipette tip to the pipette securely.

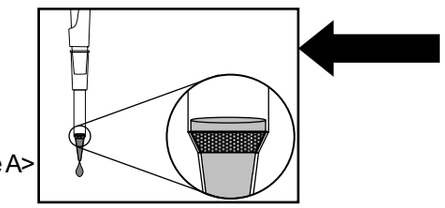
2. Dispense the preservation solution
   Depress pipette plunger to dead stop to dispense preservation solution waste.

3. Equilibration
   Aspirate 100ul of equilibration solution (50mM aqueous ammonium hydrogencarbonate) and dispense waste. Conduct this procedure twice.

4. Digestion (See Picture Right)
   Heat the protein sample solution to 37℃, and then aspirate and dispense about 20 cycles. During this procedure, do not let air reach the monolith bed (See Picture A and B)
   Caution: Use protein samples after reduction, S-alkylation and desalting.

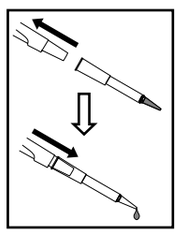


<Picture A>



5. Remove the MonoTip® Trypsin pipette tip from the pipette and soak the MonoTip® Trypsin pipette tip with the sample solution for 15-30mins.

6. Dispense the digestion solution
   Attach the MonoTip® Trypsin pipette tip to the pipette and dispense the digestion solution completely. Remove the MonoTip® Trypsin pipette tip from the pipette again and reconnect it to dispense the remaining digestion solution at the tip end (See picture E, right)



Picture E

* Some of the biotic samples (such as blood serum) might have low digestion efficiency with the MonoTip® Trypsin pipette tip. In this case, soak the sample and leave it for about 1-2 hours at 37℃ for better digestion efficiency.

6. Specifications

Description	Specifications
Processing time	Approximately 20 minutes
Loadable sample volume	20～200 μL
Tip volume	200 μL
Origin of trypsin	Cow pancreas
Organic solvent resistance	Below 20% acetonitrile
Denaturation agent resistance	Below 2 mole/L urea

7.FAQ

Equilibration solution can not be dispensed completely	Due to the tip shape, it is hard to remove the rinsing solution at the tip end completely. Remove the tip once from the pipette and attach again, then dispense.
You do not have ammonium hydrogencarbonate for equilibration solution	100mM Tris-HCl, disodium phosphate or ammonium acetate solution can be used as alternatives.
Trypsin digestion (peptide fragments) can not be obtained.	Proteins with low concentration might be absorbed into the silica monolith and unable to come out of the tip.

- The MonoTip® Trypsin is manufactured, inspected, packed and shipped under strict standards of quality control. Should you find any defect in performance, please contact us at world@gls.co.jp.
- The MonoTip® Trypsin is manufactured for the purpose of sample preparation. We do not accept any complaints when performance has deteriorated due to non-compliance with the above operating instructions.

The monolith manufacturing technology with sol-gel method was developed by Dr. N. Soga and Dr. K. Nakanishi of Kyoto University and Kyoto Monotech Co. GL Sciences Inc., Tokyo, Japan used this technology to develop and manufacture " MonoTip® ".

"Based on monolithic technology, Merck KGaA, Darmstadt, Germany"

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