MonoTip® TiO Pipette Tip

Operation Instructions

Thank you for purchasing GL Sciences' MonoTip TiO pipette tips.

The MonoTip TiO consists of a silica micro porous monolith coated with titanium dioxide and is used to enrich phosphopeptides from a peptide mixture. The MonoTip TiO shows excellent recovery of phosphopeptides, is highly reproducible, and has been a very useful tool for phosphopeptide sample preparation before mass, HPLC, LC/MS, or other subsequent analysis. To maintain optimum performance, read the following instructions before use.

1. Handling the Tip

- Do not drop or harshly tap the tips. Subjecting the tip to shocks may cause the monolith to crack and create flow around the monolith instead of through the monolith.
- Do not autoclave.

2. Using the Tip

- The volume of the MonoTip TiO is 200µL. We recommend using a Gilson Pipetman P200 or other suitable pipette.
- The MonoTip TiO is designed for the enrichment or purification of 10ug or less phosphopeptides.
- Use HPLC purity grade water, acetonitrile and formic acid to minimize organic or ionic contamination.
- The MonoTip TiO tips are designed for a single-use application. Re-use not recommended.
- When pipetting, ensure that the sample completely passes through the bed of the monolith but do not let air reach the monolith bed.
- Increasing the number of pipetting cycles during the sample "Absorption" step may improve recovery.
- Reducing the concentration of acetonitrile in the rinsing buffer (e.g. from 30% to 20%) might improve recovery of more hydrophobic phosphopeptides. Also, increasing the concentration of acetonitrile in the Elution Buffer might also help in this case.
- Higher recovery of phosphopeptides can be obtained with **Elution Buffer A** than **Elution Buffer B**. However Elution Buffer A could increase the elution of "contaminating" compounds adsorbed non-specifically to the monolith. Therefore, we recommend increasing the number of pipetting cycles when **Rinsing** to help remove non-phosphopeptides "contaminants."
- The following procedure for desalination enables direct detection of eluents by ESI negative mode.
  - After rinsing with "Rinsing Buffer, " Pipette 3 times with "Equilibration Buffer" for desalination
  - Elute with "Elution Buffer B"
- When using the MALDI-TOF/MS, neutralize the eluent with acetic acid, etc, then mix with the Matrix.

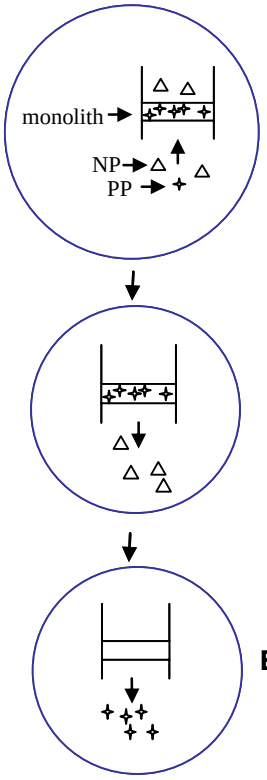
3. Typical Enrichment Protocol

1. Set the pipette volume to 200µL and secure the MonoTip TiO to the pipette.
2. **Preconditioning**. Aspirate 100% acetonitrile and then dispense. Repeat twice.
3. **Conditioning**. Aspirate **Conditioning Buffer** and then dispense. Repeat twice.
4. **Equilibration**. Aspirate **Equilibration Buffer** then dispense. Repeat twice.
5. **Adsorption**. Aspirate the sample to which 0.1 ~ 1% formic acid has been added. Dispense it back after 20 cycles of aspiration/dispensing.
6. **Rinsing**. Aspirate **Rinsing Buffer** and dispense. Repeat this procedure 6 times.
   *The higher the concentration of the salt in the rinsing buffer, the more efficient the rinsing, until the point at which phosphopeptides begin to be eluted during the rinse. For optimum enrichment, the rinsing buffer should have sufficient organic and salt concentration to remove contaminants while leaving phosphopeptides bound to the monolith surface.
7. **Elution.** In the recovery vial, prepare 50uL of an **Elution Buffer A** which elutes fewer contaminants along with the phosphopeptides. Alternatively, a salt-free **Elution Buffer B** can be used. Aspirate the elution buffer and dispense after pipetting 5 times.

4. Storage

- Store MonoTip TiO in a clean, dark, temperature-stable environment. MonoTip TiO does not need to be refrigerated.
- The MonoTip TiO is manufactured, inspected, packed and shipped under strict standards of quality control. Should you find any defect in performance, please contact us at info@glsciencesinc.com.
- The MonoTip TiO is manufactured for the purpose of sample preparation. We regret that we do not accept any claim when their performance has deteriorated due to non-compliance with the above operating instructions.
- The monolith manufacturing technology with sol-gel method was developed by Dr. Soga and Dr. Nakanishi of Kyoto University, and Kyoto Monotech Co. GL Sciences, Inc., Tokyo, Japan used this technique to develop and manufacture " MonoTip ".

	Recommended Buffer
Conditioning Buffer	0.2M Phosphate Buffer（pH 7.0）
Equilibration Buffer	0.1% Formic acid in 50% aqueous acetonitrile solution
Rinsing Buffer	0.1% Formic acid & 0.1M KCl in 50% aqueous acetonitrile solutio
Elution Buffer A	0.2M Phosphate Buffer（pH 7.0）
Elution Buffer B	0.5-5% Aqueous Ammonia



Adsorb phosphopeptide(s)

Rinse away contaminants

Elute purified phosphopeptide(s)

NP: non-phosphopeptide
PP: phosphopeptide

For additional information on how MonoTip TiO and Titansphere TiO cartridges have been successfully used to purify phosphopeptides, please visit www.glsciencesinc.com and select MonoTip TiO or Titansphere from the main menu.

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