# Welch UHPLC Column Care and Use Manual

Thank you for purchasing and using Welch UHPLC column. To prolong the life time of UHPLC column, please read this manual carefully before using.

## Column Identification:

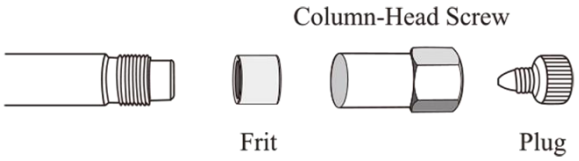
Every Welch column has a unique serial number, by which, the column can be traced back to each production procedure if any problem occurs. When customer receives the column, please check:

1. If the package is intact and the label shows the same column required.
2. If inside the box there is a CofA with a signature of quality inspector.
3. If the column has any apparent defects on surface and two end caps are complete.
4. If the column has an ID label with Welch logo and the column specification on the label is consistent with the one on the box.
5. Small particulate matter is packed inside column, please DO NOT open the column in case of inhalation. If have to, please use protection.

## Column Construction and Installation

### Structure:

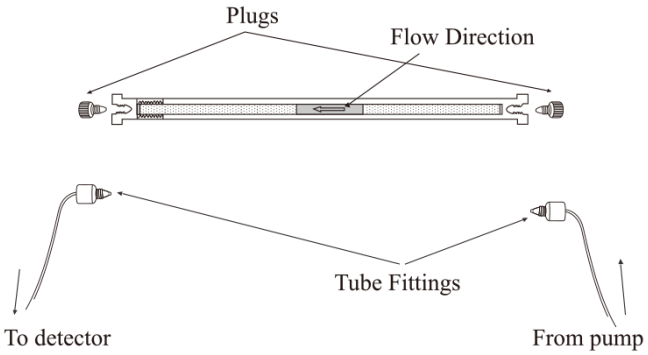
All Welch columns uses 316L stainless steel as column tube, with completely consistent both ends.




### Installation

To ensure highest column efficiency, use connection tubes with matched diameter. The connection of both ends must be highly fitted. The tube ends must be smooth, without any burrs or slants. Use professional tools to cut the tubes.

1. Find an arrow on the column identification label which indicates the correct direction of solvent flow. Unscrew the caps of each ends and place it in the right direction.




2. Switch the column using matched connectors (stainless steel or other materials). Make sure the column is tightly connected, without any dead volume or leaking when opening.

## Column parameters

| Column | USP | Carbon Load | pH Range | Max. Temp. |
|---|---|---|---|---|
| Xtimate® UHPLC C18 | L1 | 14% | 1.0-12.5 | 70℃(pH≤6.5) |
| Ultisil® UHPLC LP-C18 | L1 | 10% | 0.5-8.0 | 40℃(pH＞6.5) |
| Ultisil® UHPLC XB-C18 | L1 | 17% | 1.5-10.0 | |
| Ultisil® UHPLC AQ-C18 | L1 | 12% | 1.5-10.0 | |
| Ultisil® UHPLC Polar-RP | L1 | 18% | 1.5-10.0 | 60℃(pH≤6.5) |
| Ultisil® UHPLC HILIC | L3 | N/A | 2.0-8.0 | 40℃(pH＞6.5) |
| Ultisil® UHPLC XB-C8 | L7 | 12% | 1.5-10.0 | |
| Ultisil® UHPLC XB-Phenyl | L11 | 12% | 1.5-10.0 | |

Pore Size: 120 Å  Surface Area: 320$m^2$/g  Particle Size: 1.8μm
Max. Pressure: 15000 psi

## Activation and Transition

| | |
|---|---|
| Phases | Xtimate® UHPLC C18, Ultisil® UHPLC XB-C18, Ultisil® UHPLC AQ-C18, Ultisil® UHPLC LP-C18, Ultisil® UHPLC Polar-RP, Ultisil® UHPLC XB-C8, Ultisil® UHPLC XB-Phenyl |

**Storage Solvent: methanol/water**

| | Activation | | Transition | |
|---|---|---|---|---|
| Column ID | ≤3mm | >3mm | ≤3mm | >3mm |
| Flow rate | 0.1ml/min | 0.2ml/min | Analysis flow rate | |
| Mobile phase | 80% methanol | | 10% methanol* | |
| Time | 4h | | 1h | |
| Temperature | 30℃ | | 30℃ | |
| Note | No Transition required for analysis mobile phases without buffer. | | | |

*Transitional mobile phase refers to the proportion between organic phase and aqueous phase which is the same as that of analytical mobile phase, except that there is no buffer salt. 10% methanol is recommended as transitional mobile phase in most instances.

## Column Daily Flushing

**Back flushing recommended** (reverse to the normal flow direction)

| Analysis Mobile Phase | Without acid, alkali or salts | Containing acid, alkali or salts | Containing ion-pair reagents |
|---|---|---|---|
| Flow rate | Analysis flow rate | | |
| Flushing Mobile Phase | 80%methanol | 10%methanol - 80%methanol | 10%methanol - 50%methanol - 80%methanol |
| Time | Column length≤100mm, 30min each step | | |
| | Column length>100mm, 40min each step | | |
| Storage | Store in the last flushing solvents, kept in cool dry places | | |
| Note | The methanol in the flushing mobile phase can be changed into acetonitrile. | | |

## Abnormal Column Flushing

In circumstances of high column pressure, abnormal peak shape, low column efficiency or low resolution etc., use transition mobile phase to flush off the salts in the column, then flush as following; If mobile phase contains ion-pair reagents, use 50% methanol in the first step and flush as following:

**Back flushing recommended** (reverse to the normal flow direction)

| | |
|---|---|
| Flow Rate | 1/4 analysis flow rate |
| Mobile Phase | 100% methanol-100% acetonitrile-<br>100% isopropanol- 100%acetonitrile |
| Time | Column length≤100mm, 100min each step |
| | Column length>100mm, 120min each step |
| Note | Isopropanol has high viscosity, causing high pressure. Please adjust the flow rate as needed. |

| | |
|---|---|
| Phase | Ultisil® UHPLC HILIC |

**Storage Solvent: acetonitrile**

| | Activation | | Transition | |
|---|---|---|---|---|
| Column ID | ≤3mm | >3mm | ≤3mm | >3mm |
| Flow rate | 0.1ml/min | 0.2ml/min | Analysis flow rate | |
| Mobile phase | 100% acetonitrile | | Transitional mobile phase* | |
| Time | 4h | | 1h | |
| Temperature | 30℃ | | 30℃ | |
| Note | No Transition required for analysis mobile phases without buffer. | | | |

*Transitional mobile phase refers to the proportion between organic phase and aqueous phase which is the same as that of analytical mobile phase, except that there is no buffer salt.

## Column Daily Flushing

**Back flushing recommended** (reverse to the normal flow direction)

| | | |
|---|---|---|
| Analysis Mobile Phase | Without acid, alkali or salts | Containing acid, alkali or salts |
| Flow rate | Analysis flow rate | |
| Flushing Mobile Phase | 100% acetonitrile | Transitional mobile phase -100% acetonitrile |
| Time | Column length≤100mm, 30min each step | |
| | Column length>100mm, 40min each step | |
| Storage | Store in pure acetonitrile; Kept in cool dry places | |

## Abnormal Column Flushing

In circumstances of high column pressure, abnormal peak shape, low column efficiency or low resolution etc., use transition mobile phase to flush off the salts in the column, then flush as following; If mobile phase contains ion-pair reagents, use 50% methanol in the first step and flush as following:

**Back flushing recommended** (reverse to the normal flow direction)

| | |
|---|---|
| Flow Rate | 1/4 analysis flow rate |
| Mobile Phase | 100% methanol -100% acetonitrile-<br>100% isopropanol - 100% acetonitrile |
| Time | Column length≤100mm, 100min each step |
| | Column length>100mm, 120min each step |
| Note | Isopropanol has high viscosity, causing high pressure. Please adjust the flow rate as needed. |

## Precautions

1. It is recommended to filter the sample and mobile phase with 0.22μm membrane before testing.
2. Inline filter is recommended when using UHPLC column to prolong column lifetime.

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