Prep Column User Manual

Thank you for choosing Welch column. Please read the user instructions carefully before using.

COLUMN IDENTIFICATION

Each column comes with its own serial number. The serial number allows us to track the manufacturing history of each column so that we can guarantee its quality. To better serve you, please validate the identity of the column and its quality upon reception by checking the following items:

- Intact column box, with correct label
- Column QA performance report bearing certification signature inside
- Intact column body, sealed with caps on both ends
- Column label contains same information as box label, including model, part number and serial number. If there is any question or problem regarding the quality of the column, please feel free to contact us directly.

PRECAUTIONS FOR INSTALLATION

1. Avoid any impact when taking or moving Prep column. Damage to the column bed would be irreversible and greatly affect column performance.
2. Use 1/16 or 1/8 stainless steel or PEEK tubings to connect the column, to minimize dead volume. Columns of 50mm or larger ID use 1/8 connector(normally attached with a 1/16 adapter).
3. Ensure the tube and fittings are properly connected and tightened.
4. If any joint between tube and fitting leaks, try check the type and size of tubings and reconnect.
5. If any joint between column and fittings leaks, try tighten the fittings clockwise with a wrench. DONOT over tighten the fitting, especially stainless steel fittings, to avoid jamming.
6. Ensure the mobile phase flows in the same direction as showed on column body.

COLUMN EQUILIBRATION

Unless specifically noted, mobile phase used on our prep column shall be same as stated on column test reports. So please make sure mobile phases used are mutually soluble with factory mobile phase.
Reversed Phases including ODS(C18), Octyl(C8), Phenyl, Butyl(C4) and Cyano(CN), shall use water-soluble organic solvents.
Normal Phases shall use solvents like n-hexane, dichloromethane, trichloromethane and isooctane etc.
Please use at least 10 column volumes of mobile phase to equilibrate the column.
Prep column volume calculating formula: $V=\pi r^2 L$
(V=column volume; π=3.1415926; r=column radius/cm; L=column length/cm)

Column Volumes of Normal Column Sizes

Column Dimension	Column Volume	Minimum Equilibration Volume
10×250mm	19.6ml	196ml
21.2×250mm	88.2ml	882ml
30×250mm	176.7ml	1767ml
50×250mm	490.8ml	4908ml

1. Connect the column inlet with injection valve outlet after purge.
2. Connect the column outlet with detector inlet when mobile phase flows evenly out of column, thus to avoid air into detector and decrease equilibrating time.
3. When mobile phase changes, slowly increase flow rate.
4. Column shall be well equilibrated and ready for testing when column pressure and baseline go steady.

Note: If mobile phase contains low-concentration additives (e.g. 5-10mmol/L ion-pair reagents), column shall be equilibrated with 100-200 column volumes of mobile phase.

SAMPLE PREPARATION

1. It is suggested to dissolve sample with mobile phase or weaker solvents.
2. If sample does not dissolve in mobile phase, ensure the dissolving solvents is mutually soluble with mobile phase and sample, to avoid precipitation.
3. Filtrate sample solution with 0.22μm membrane.

PRECAUTIONS FOR USE

1. Guard Column

Impure Sample brings impurities that may pollute the column and decrease column performance and lifetime.
A: Use SPE cartridge for sample pretreatment
B: Use Guard Column to filtrate those impurities in sample solution and mobile phase that cannot be pretreated, thus to avoid blocking sieve plate, causing high column pressure. Guard column used shall have same physico-chemical properties like prep column.

2. pH Range

Each prep column has its own specific working pH range. Using column under pH out of range may cause irreversible damage like dissolution of silica base and hydrolyzation of bonding phase. When working pH is near the limit, please use mobile phase that contains at least 10% of organic solvents. Using column under critical pH may decrease column lifetime, so solvents inside column shall be replaced with eluents which is soluble with mobile phase and suitable for column storage.

Prep Column Reference pH Range

Column	C1, C4, C8, C18	Phenyl	CN
pH Range	1.5-10.0	1.5-10.0	1.5-9.0
Column	LP-C18, LP-C8	AQ-C18	Xtimate Series
pH Range	0.5-8.0	1.5-10.0	1.0-12.5

3. Reagents

High-grade chromatographically pure reagents are suggested for better performance. Each reagent shall be well filtrated before using, to avoid some suspended particles going to the column inlet. Reagents used for mobile phase shall be pre-degassed, to avoid any bubbles into the system.

4. Pressure

Column back pressure is concerned with: 1. Particle size and distribution; 2. Column dimension; 3. Reagents viscosity, flow rate and temperature.

Change of flow rate shall be slow and steady, avoiding rapid change of pressure, thus to protect the column and extend lifetime.

Particle Size	5μm	10μm	15μm	20μm	20-40μm	40-70μm
Max. Pressure	40Mpa	25Mpa	15Mpa	10Mpa	5Mpa	4Mpa

5. Column Temperature

Suggested column temperature range is 30-50℃. Suitable temperature will decease reagent viscosity, and increase column selectivity and reproducibility.

STORAGE

1. DONOT store column with mobile phase that contains buffer salts, acids or bases.
2. After using mobile phase containing buffer salts or salts, flush the column following column rinsing procedures and replace the solvents with factory mobile phase for storage.
3. Ensure both caps on column ends are tightened, avoiding liquid volatilization and base material drying up.

Note: Each Column shall be equipped with caps on both ends

FLOW RATE & SAMPLE LOADING

Operating flow rate, sample loading and column size shall obey following rules:

A. Proportional to Flow rate and Column radius: $F_2=F_1(r_2/r_1)^2$

B. Retention time stays still: $F_2=F_1(L_2/L_1)(r_2/r_1)^2$

C. Sample Loading: $W_2=W_1x(L_2/L_1)(r_2/r_1)^2$

D. Amplification Factor=$(L_2/L_1)(r_2/r_1)^2$

(L=column length; r=column radius; F=flow rate(ml/min); W=sample loading; 1=previous column; 2=replace column)

Amplification Reference

Column Size	4.6×250 mm	10×250 mm	21.2×250 mm	30×250 mm	50×250 mm
Packing Media(g)	2.5	11.8	53.1	106.3	295.4
Amplification Factor	1	4.73	21.2	42.5	118
Sample(mg)	0.25-25	1.18-118	5.31-531	10.63-1063	29.54-2954
Flow Rate(ml/min)	0.5-2	3-5	10-20	20-45	70-130

MAINTENANCE

Column performance decreases by the time it has been used. Replacing a new column is suggest for situations like peak expanding, low resolution and high column pressure etc.

1. Increasing Column Pressure

A slowly increasing column pressure due to long-term using is normal. If column pressure increases suddenly or in a short time, excluding the problem of the system, try check the problems and suggestions as following:

A. Polluted sieve plate in column head

Suggestion: For complicated samples with small particle impurities, try adding an in-line filter or guard column before prep column; For jammings of some insolubles, try replacing proper solvents or membrane filtrating sample before injection; For polluted column head, try reversely rinsing prep column with 20-30 column volumes in low flow rate. If reverse rinsing does not help, please contact us for help.

B. Polluted packing media in column head

Accumulation of pollutants due to long-term using may cause increase of column pressure.

Suggestion: Try reversely rinsing prep column in low flow rate with 20-30 column volumes of solvents that can dissolve pollutants. If reverse rinsing does not help, please contact us for help or replace the prep column.

C. Improper pH condition

Suggestion: Improper pH conditions used may cause loss of stationary phase, for which prep column can not be repaired. Please replace with a new column.

2. Buffer Salts Precautions

As buffer salts are normally soluble in water and insoluble in organic solvents, high-ratio organic solvents used may cause salting out, and subsequently the salted-out particles will accelerate the wear of system parts, flow into column and block inlet sieve plate and even packing media, causing increase of column pressure and decrease of column efficiency and lifetime. As salted-out buffer salts are difficult to remove, please check the following notes to avoid salting out:

A. Isocratic: Flush column with at least 20-30 column volumes of transition mobile phase before and after using the column; Or after using, flush overnight with transition mobile phase in 2ml/min flow rate.

(**Transition Mobile Phase:** with same ration of organic phase and water phase as analytical mobile phase, with higher water content; absolutely without buffer salts)

B. Gradient: Before using, flush with at least 20-30 column volumes of mobile phase(same as original mobile phase) in analytical flow rate. After using, flush with at lease 20-30 column volumes of transition mobile phase. Gradient change shall be as steady as possible to avoid salting out.

C. In case of buffer salted out: Reversely flush column with 20-30 column volumes of Methyl/Water(10/90) in analytical flow rate under 35℃; Or reversely flush overnight with 20-30 column volumes of Methyl/Water(10/90) in 2ml/min flow rate. If both procedures do not help, please contact us for help.

3. Column Rinsing

The accumulation of strong-retention substances and macromolecular compounds inside column is a rather slow process, which will bring strong retention to sample contents, causing peak widening and efficiency decrease. To avoid this, routine column rinsing and maintenance is needed:

A. If mobile phase does not contain buffer salts or salts material, use high-ratio organic phase solvents to flush off strong-retention substances, or use 100% organic phase. For high-liposolubility contents, try adding 10% THF to get better removal efficiency.

B. If mobile phase contains buffer salts or salts material, please refer to Buffer Salts Precautions and flush the column.

C. If macromolecules like proteins and polysaccharides are adsorbed in column, try flushing the column with Acetonitrile/Water/TFA(50/50/0.1). If sample contains such substances or too much impurities, please do sample pretreatment before injection.

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