

BIOSUITE COLUMNS

I. INTRODUCTION

Thank you for choosing a Waters BioSuite™ column. Every column is individually tested to assure maximum product quality. Waters BioSuite high performance chemistries are dedicated to the isolation, analysis, and characterization of proteins and peptides. For the latest list on ion-exchange, size exclusion, hydrophobic interaction and reversed-phase go to www.waters.com/lifesciences

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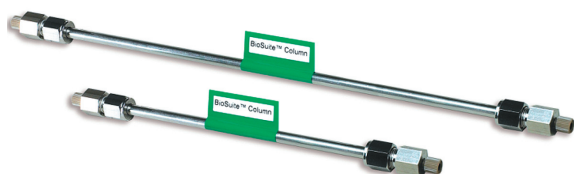
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BioSuite™
Columns

a. Mobile Phase

Prevent air bubbles from entering the column during its installation, use, and storage since this may cause degradation of column performance through the formation of channels in the packed bed. Mobile phases must be thoroughly degassed before use. This can be accomplished by vacuum filtration, helium sparging or by in-line vacuum degassing. In addition to degassing the solvent, vacuum filtration of the solvent will also prevent small particles from plugging the column frit. You can use 0.20 or 0.45 µm membrane to filter aqueous and aqueous/organic mobile phases. (Note: Consult with filtration membrane manufacturer for details on solvent compatibility.)

Note: Use high quality reagents, HPLC grade water, and HPLC grade solvents for preparing buffers to maximize column efficiency especially when using Waters BioSuite Non-Porous (NP) columns.

The useful column lifetime is a function of numerous factors including: the cleanliness and composition of the mobile phase and the sample; the flow rate and pressure used; and the temperature. Refer to the section below about “Cleaning” for information on extending column life.

Note: Cleaning, however, is not effective when the column is damaged by irreversible sample adsorption, channeling, or exposure of the packing material to excessive heat or shock.

b. Flow Direction

The recommended flow direction through the column is indicated by the arrow on the tag. Operating the column with the flow in the reverse direction is only recommended as part of a cleaning procedure when removing particulates from a clogged frit.

II. CONNECTING YOUR COLUMN TO THE HPLC SYSTEM

Due to the absence of an industry standard, various column manufacturers have employed different styles of chromatographic column connectors. The chromatographic performance of your separation can be negatively affected if the style of column end fitting does not match the existing instrumentation tubing ferrule setting. Waters BioSuite columns require end fittings that have a 0.175” depth between the ferrule and the end of the extending stainless steel tubing (Figure A) that must perfectly seat to the end of the BioSuite column. Connecting a non-BioSuite style connector to a Waters BioSuite column will leave a gap between the end of the stainless steel

tubing and the column that will result in undesired peak broadening (Figure B). To correct this problem, Cut the tubing, place a new ferrule on it and re-make the connection (i.e., 0.175” depth) to the Waters BioSuite column.

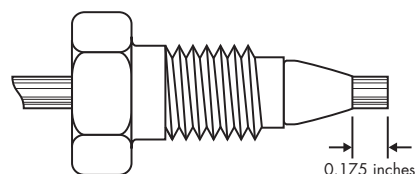


Figure A

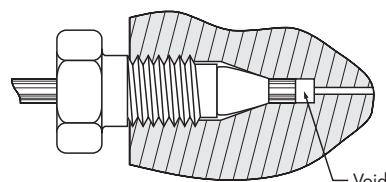


Figure B

In a proper tubing/column connection, the tubing touches the bottom of the column end fitting with no void between them. (Figure C)

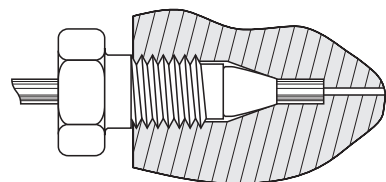


Figure C

After manufacturing and quality control, the column has been flushed with storage solvent and closed with caps to prevent solvent evaporation. When installing the column to the Liquid Chromatograph, it is important to prevent air from entering the column. The following steps can be used to minimize this potential problem:

- Remove the cap from the column's inlet side. Solvent should be visible at the inlet fitting (if not, see below).
- Start solvent flow through the liquid chromatograph BEFORE connecting the Waters BioSuite column to the system. Turn off the pump after a steady flow of solvent from the system is observed. You can now connect the waters BioSuite column to the liquid chromatograph and slowly begin delivering solvent to the column (Note: If the Waters BioSuite column inlet fitting appears dry, we recommend that you first disconnect the bottom cap and hook up the column exit to the injector. Then slowly start

the flow in this reversed flow direction until a few drops of mobile phase exit from the column. Turn off the flow, let the pressure go to zero, and disconnect the column from the system. Turn the column around and hook it up so that the flow is now in the direction of the arrow. Start the flow at a low setting and stop it as soon as the mobile phase exits from the bottom fitting. Now you can hook up the column to the detector inlet and increase the flow to the desired setting.)

- Be sure to set the flow within the recommended range shown in the following tables.

Sample

If possible, always dissolve your sample in mobile phase or the starting mobile phase when operating under gradient conditions. Alternatively, try to match the pH, salt concentration and organic solvent of the sample with those of the mobile phase and run a test to ensure that no precipitate, suspension or flocculate is formed. Finally, before making an injection, filter the sample through a 0.20-0.45 µm porosity membrane.

III. WATERS BIOSUITE PC₁₈ AND PPHENYL RPC COLUMNS

This section contains recommended operating conditions and specifications for the Waters polymer-based, C₁₈ and phenyl reversed-phase columns.

Operating Conditions

1. Shipping Solvent: 70% Methanol - 30% Water
2. Recommended Operating Flow Rates/Max Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite pC ₁₈ , 2.5 µm NP RPC	polymer	4.6	35	1.0 - 1.6 mL/min / 3000 PSI
BioSuite pC ₁₈ , 500, 7 µm RPC	polymer	4.6	150	0.08 - 0.22 mL/min / 1500 PSI
BioSuite pC ₁₈ , 500, 7 µm RPC	polymer	4.6	150	0.5 - 1.2 mL/min / 1750 PSI
BioSuite pC ₁₈ , 500, 13 µm RPC	polymer	21.5	150	3.0 - 8.0 mL/min / 500 PSI
BioSuite pPhenyl, 1000, 10 µm RPC	polymer	2.0	75	0.05 - 0.12 mL/min / 150 PSI
BioSuite pPhenyl, 1000, 10 µm RPC	polymer	4.6	75	0.5 - 1.2 mL/min / 450 PSI
BioSuite pPhenyl, 1000, 13 µm RPC	polymer	21.5	150	6.0 - 8.0 mL/min / 450 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate (20% for the 21.5 mm i.d. preparative column).

3. Recommended pH Range: 2 - 12 (Avoid extended operation at pH > 10 to maximize column life.)
4. Recommended Organic Concentration Range: 0 - 100% (Note: Reduced organic concentrations may be required if buffers are present in the eluent in order to avoid salt precipitation.)
5. Recommended Temperature Range: 5 - 50 °C (Note: Reduce flow rate when operating at low temperatures (e.g., 5 C) to avoid excessive column pressure.)
6. Recommended Cleaning Solvents/Procedures:
 - A) Acetonitrile or Methanol, or
 - B) Aqueous buffer in organic solvent, or
 - C) 0.1 - 0.2M NaOH, or
 - D) 20 - 40% acetic acid in HPLC grade water.

Clean the column regularly with any of the listed solvents. Cleaning solvents (C) and (D) can be injected onto the column in 250 µl increments for a total of one column volume. Use proportionally larger volumes for the 21.5 mm i.d. prep column. Following use of cleaning solvent, rinse the column with 3 to 5 column volumes of HPLC grade water before storage.

7. Recommended Storage: For overnight storage, continuously flush the column with the mobile phase at 10 - 20% of the maximum recommended flow rate. Store the column in the shipping solvent when the column will not be used within 48 hours.

IV. WATERS BIOSUITE PHENYL HIC COLUMNS

This section contains recommended operating conditions and specifications for the Waters polymer-based, Phenyl Hydrophobic-interaction Chromatography columns.

Operating Conditions

- Shipping Solvent: HPLC grade Water
- Recommended Operating Flow Rates/Max Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite Phenyl, 1000, 10 µm HIC	polymer	7.5	75	0.5 - 1.2 mL/min / 300 PSI
BioSuite Phenyl, 1000, 13 µm HIC	polymer	21.5	150	4.0 - 8.0 mL/min / 300 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate (20% for the 21.5 mm i.d. preparative column).

- Recommended pH Range: 2 - 12 (Avoid extended operation at pH > 10 to maximize column life.)
- Recommended Organic Conc.: less than 50% (Note: Reduced organic concentrations may be required if buffers are present in the eluent in order to avoid salt precipitation.)
- Recommended Salt Conc.: Less than or equal to 4.0 Molar
- Recommended Temperature: 10-50 °C. Reduce flow rate when operating at low temperatures (e.g. 10 °C) to avoid excessive column pressure.
- Recommended Cleaning Solvents/Procedures:
 - 0.1 - 0.2M NaOH, and, if not successful,
 - 20 - 40% acetic acid in HPLC grade water.

Clean the column regularly by injecting up to one column volume 0.1 - 0.2M NaOH in 250 µl increments. Use proportionally larger volumes for the 21.5 mm i.d. preparative column. Following use of cleaning solvent, rinse the column with 3 to 5 column volumes of HPLC grade water before storage.

- Recommended Storage: For overnight storage, continuously flush the column with the mobile phase at 10 - 20% of the maximum recom-

mended flow rate. Store the column in the HPLC grade water when it will be used within 24 hrs or in either 20% ethanol or methanol for long term storage. (Caution: Rinse column in HPLC grade water before switching to 20% organic solvent to avoid salt precipitation in column.)

V. WATERS BIOSUITE ULTRA-HIGH RESOLUTION (UHR), HIGH RESOLUTION (HR), AND SIZE-EXCLUSION (SEC) COLUMNS

This section contains recommended operating conditions and specifications for the Waters silica-based, ultra-high Resolution (UHR), high-resolution (HR), and standard size-exclusion (SEC) columns.

Operating Conditions

- Shipping Solvent: 0.05% Sodium Azide or 0.1M Sodium Sulphate in 0.1M phosphate buffer, pH 6.7
- Recommended Operating Flow Rate/Max Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite 125, 4 µm UHR SEC	silica	4.6	300	0.1 - 0.4 mL/min / 1800 PSI
BioSuite 250, 4 µm UHR SEC	silica	4.6	300	0.1 - 0.4 mL/min / 1800 PSI
BioSuite UHR Guard SEC	silica	4.6	35	0.1 - 0.4 mL/min / 1800 PSI
BioSuite 125, 5 µm HR SEC	silica	7.8	300	0.5 - 1.2 mL/min / 1050 PSI
BioSuite 250, 5 µm HR SEC	silica	7.8	300	0.5 - 1.2 mL/min / 1050 PSI
BioSuite 450, 8 µm HR SEC	silica	7.8	300	0.5 - 1.2 mL/min / 525 PSI
BioSuite HR Guard SEC	silica	6	40	0.5 - 1.2 mL/min / 1050 PSI
BioSuite 125, 10 µm SEC	silica	7.5	300	0.5 - 1.2 mL/min / 300 PSI
BioSuite 125, 13 µm SEC	silica	21.5	300	3.0 - 8.0 mL/min / 150 PSI
BioSuite 250, 10 µm SEC	silica	7.5	300	0.5 - 1.2 mL/min / 375 PSI

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite 250, 13 µm SEC	silica	21.5	300	3.0 - 8.0 mL/min / 225 PSI
BioSuite 450, 13 µm SEC	silica	7.5	300	0.5 - 1.2 mL/min / 225 PSI
BioSuite 450., 17 µm SEC	silica	21.5	300	3.0 - 8.0 mL/min / 300 PSI
BioSuite Guard SEC	silica	7.5	75	0.5 - 1.2 mL/min / 600 PSI
BioSuite Guard SEC	silica	21.5	75	3.0 - 8.0 mL/min / 450 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate.

- Recommended pH Range: 2.5 - 7.5
- Recommended Salt Conc.: Less than or equal to 0.5 Molar
- Recommended Organic Conc.: 0 - 100% (Caution: Many proteins and peptides are insoluble at elevated organic concentrations. Prior to chromatography, test to ensure the sample does not precipitate at the organic concentration to be used for the chromatography.)
- Recommended Temperature: 10-30 °C. Reduce flow rate when operating at low temperatures (e.g., 10 °C) to avoid excessive column pressure.
- Recommended Cleaning Solvents/Procedures:
 - A concentrated salt solution at low pH, e.g. 0.5M Na₂SO₄, pH 2.7
 - A low concentration of methanol or acetonitrile (e.g., 20%) in HPLC grade water
 - A buffered solution of SDS, urea, or guanidine

Note: Choose a cleaning solvent based on sample properties, e.g. use (A) to remove basic proteins, and (B) to remove hydrophobic proteins. Chaotropic agents can solvate strongly adsorbed proteins via hydrogen bond disruption.

- Recommended Storage: For overnight storage, continuously flush the column with the mobile phase at 10 - 20% of the maximum recommended flow rate. Store the column in the HPLC grade water when it will be used within 24 hrs or in either 20% ethanol or methanol for long term storage. (Caution: Rinse column in HPLC grade water

before switching to 20% organic solvent to avoid salt precipitation in column.)

- Recommended Column Protection: Use of guard columns is recommended to prolong the life of the analytical column life. Guard column life depends greatly on sample cleanliness. As a general rule, guard columns should be replaced after every 30-40 sample injections, when the peaks become excessively wide, or when the peaks show splitting.

VI. WATERS BIOSUITE Q-PEEK AND SP-PEEK COLUMNS

This section contains recommended operating conditions and specifications for the Waters polymer-based, anion and cation-exchange materials contained in PEEK™ columns.

Note: Compared to use of stainless-steel columns, connect the PEEK column to the HPLC or Liquid Chromatographic System with a 10-32 polymer nut and ferrule.

Operating Conditions:

- Shipping Solvent: 20% Ethanol containing 20 mM Tris-HCl buffer (pH 8.0)
- Recommended Operating Flow Rates/Max. Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite Q-PEEK 10 µm / AXC	polymer	4.6	50	0.3 - 1.2 mL/min / 375 PSI
BioSuite SP-PEEK 7 µm / CXC	polymer	4.6	50	0.3 - 1.0 mL/min / 375 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate.

- Recommended pH Range: 2 - 12 (Avoid extended operation at pH values less than 3 or greater than 10 to maximize column life.)
- Recommended Salt Conc.: Less than or equal to 2.5 Molar
- Recommended Organic Conc.: 0 - 30%. (Caution: Some large molecular weight proteins may be insoluble at elevated organic

concentrations. Prior to chromatography, test to ensure the sample does not precipitate at the organic concentration to be used for the chromatography.)

6. Recommended Temperature: 4 - 60 °C. Reduce flow rate when operating at low temperatures (e.g., 4 °C) to avoid excessive column pressure.
7. Recommended Cleaning Solvents/Procedures:
 - A) 0.1 - 0.5M NaOH in HPLC grade water and if not successful,
 - B) 20 - 40% acetic acid in HPLC grade water or,
 - C) 30% acetonitrile or 30% methanol in HPLC grade water or,
 - D) 30% ethanol containing 0.5M NaOH or,
 - E) 8M Urea, 6M Guanidine or other nonionic surfactant HPLC grade water

Clean the column regularly by injecting up to one column volume of solution (A) (i.e., 0.1 - 0.5M NaOH in HPLC grade water) in 100 to 250 microliter increments. To maximize cleaning effect, reverse the direction of eluent flow through the column and reduce the flow rate by 25%.

If desired, the HPLC column can also be cleaned by delivering, in reverse eluent flow direction and at a 25 % standard flow rate, one of the alternative cleaning solvents (i.e., 20 - 40% acetic acid in HPLC grade water, or 30% acetonitrile or methanol in HPLC grade water, or 30% ethanol containing 0.5M NaOH, or either 8M Urea, 6M Guanidine or other nonionic surfactant in HPLC grade water).

8. Recommended Storage: For overnight storage, continuously flush the column with the mobile phase at 10 - 20% of the maximum recommended flow rate. Store the column in the HPLC grade water when it will be used within 24 hrs or in 20% ethanol containing 20mM Tris-HCl buffer, pH 8 for long term storage.

VII. WATERS BIOSUITE DEAE AND SP NP COLUMNS

This section contains recommended operating conditions and specifications for the Waters non-porous, polymer-based, anion-and cation-exchange columns.

Operating Conditions:

1. Shipping Solvent: The columns are shipped in HPLC grade water. Upon receiving, flush the column with 30% acetonitrile/70% HPLC grade water for 15 minutes at 1 mL/min. This is followed by equilibrating the columns with the starting buffer.

Use high quality reagents, HPLC grade water, and HPLC grade solvents for preparing buffers to maximize Waters BioSuite non-porous (NP) column efficiency and life. Filter all eluents through a 0.20 or 0.45 µm membrane before use (Consult the with filtration membrane manufacturer for details on solvent compatibility.) Fouling of the non-porous resin, leading to a loss in retention and/or efficiency, occurs faster compared to use of porous packing materials due to the small surface area of non-porous resin particles. The useful column lifetime is a function of numerous factors including the cleanliness and composition of the mobile phase and the sample, the flow rate and operating temperature, and the operating pressure. While column cleaning is possible (see below), cleaning is not effective when the column is damaged by irreversible sample adsorption, channeling, or exposure of the packing material to excessive heat of shock.

2. Recommended Operating Flow Rates/Max Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite DEAE 2.5 µm NP AXC	polymer	4.6	35	1.0 - 1.6 mL/min / 3000 PSI
BioSuite SP 2.5 µm NP CXC	polymer	4.6	35	1.0 - 1.6 mL/min / 3000 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate.

3. Recommended pH Range: 2 - 12 (Avoid extended operation at pH values less than 3 or greater than 10 to maximize column life.)
4. Recommended Salt Conc.: Less than or equal to 1.0 Molar
5. Recommended Organic Conc.: 0 - 20%.

6. Recommended Temperature: 4 - 60 °C. Reduce flow rate when operating at low temperatures (e.g., 4 °C) to avoid excessive column pressure.

7. Recommended Cleaning Solvents/Procedures:

- A) 0.1 - 0.2M NaOH in HPLC grade water and if not successful,
- B) 20 - 40% acetic acid in HPLC grade water or,
- C) 30% acetonitrile, 30% methanol, or 30% ethanol in HPLC grade water or,
- D) 8M Urea, 6M Guanidine or other nonionic surfactant HPLC grade water

Clean the column regularly by injecting up to one column volume of solution (A) (i.e., 0.1 - 0.2M NaOH in HPLC grade water) in 100 to 250 microliter increments. To maximize cleaning effect, reverse the direction of eluent flow through the column and reduce the flow rate by 25%.

If desired, the HPLC column can also be cleaned by delivering, in reverse eluent flow direction and at a 25% standard flow rate, one of the alternative cleaning solvents (i.e., 20 - 40% acetic acid in HPLC grade water, or 30% acetonitrile, 30% methanol, or 30% ethanol in HPLC grade water, or either 8M Urea, 6M Guanidine or other nonionic surfactant in HPLC grade water).

8. Recommended Storage: For overnight storage, continuously flush the column with the mobilephase at 10 - 20% of the maximum recommended flow rate. Store the column in the HPLC grade water when it will be used within 24 hrs or in 20% acetonitrile in HPLC grade water for long term storage.

VIII. WATERS BIOSUITE Q, DEAE, SP, AND CM COLUMNS

This section contains recommended operating conditions and specifications for the Waters polymer-based, anion- and cation-exchange columns.

Operating Conditions:

1. Shipping Solvent: HPLC grade Water
2. Recommended Operating Flow Rate/Max Pressure:

Description	Matrix	i.d. (mm)	L (mm)	Flow Rates / Max Pressure
BioSuite Q 10 µm AXC	polymer	7.5	75	0.5 - 1.2 mL/min / 300 PSI
BioSuite Q 13 µm AXC	polymer	21.5	150	4.0 - 8.0 mL/min / 300 PSI
BioSuite DEAE 10 µm AXC	polymer	7.5	75	0.5 - 1.2 mL/min / 225 PSI
BioSuite DEAE 13 µm AXC	polymer	21.5	150	4.0 - 8.0 mL/min / 375 PSI
BioSuite SP 10 µm CXC	polymer	7.5	75	0.5 - 1.2 mL/min / 225 PSI
BioSuite SP 13 µm CXC	polymer	21.5	150	4.0 - 8.0 mL/min / 375 PSI
BioSuite CM 10 µm CXC	polymer	7.5	75	0.5 - 1.2 mL/min / 225 PSI
BioSuite CM 13 µm CXC	polymer	21.5	150	4.0 - 8.0 mL/min / 375 PSI

Note: When using a high viscosity buffer and ambient temperature, the maximum flow rate may have to be reduced to avoid exceeding the maximum recommended operating pressure for the column. When changing solvents, use 50% of the maximum flow rate (20% for the 21.5 mm i.d. preparative column).

3. Recommended pH Range: 2 - 12 (Avoid extended operation at pH values less than 3 or greater than 10 to maximize column life.)
4. Recommended Salt Conc.: Less than or equal to 1.0 Molar
5. Recommended Organic Conc.: 0 - 20%.
6. Recommended Temperature: 4 - 40 °C. Reduce flow rate when operating at low temperatures (e.g., 4 °C) to avoid excessive column pressure.
7. Recommended Cleaning Solvents/Procedures:
 - A) 0.1 - 0.2M NaOH in HPLC grade water and if not successful,
 - B) 20 - 40% acetic acid in HPLC grade water, or

- C) 30% acetonitrile, 30% methanol, or 30% ethanol in HPLC grade water, or
 D) 8M Urea, 6M Guanidine or other nonionic surfactant HPLC grade water

Clean the column regularly by injecting up to one column volume of solution (A) (i.e., 0.1 - 0.2M NaOH in HPLC grade water) in 250 microliter to 2 mL increments. To maximize cleaning effect, reverse the direction of eluent flow through the column and reduce the flow rate by 25%.

If desired, the HPLC column can also be cleaned by delivering, in reverse eluent flow direction and at a 25% standard flow rate, one of the alternative cleaning solvents (i.e., 20 - 40% acetic acid in HPLC grade water, or 30% acetonitrile, 30% methanol, or 30% ethanol in HPLC grade water, or either 8M Urea, 6M Guanidine or other nonionic surfactant in HPLC grade water).

8. **Recommended Storage:** For overnight storage, continuously flush the column with the mobile phase at 10 - 20% of the maximum recommended flow rate. Store the column in the HPLC grade water when it will be used within 24 hrs or in 20% acetonitrile in HPLC grade water for long term storage.

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 43 1 877 18 07

Australia 61 2 9933 1777

Belgium 32 2 726 1000

Brazil 55 11 4134 3788

Canada 1 800 252 4752 x2205

China 86 21 6879 5888

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