Silica-based:

TSKgel Protein C_4-300	TSKgel ODS-140HTP	TSKgel ODS-100V
TSKgel ODS-100Z	TSKgel Super-ODS	TSKgel Super-Octyl
TSKgel Super-Phenyl	TSKgel CN-80Ts	TSKgel Octyl-80Ts
TSKgel ODS-80TM	TSKgel ODS-80Ts	TSKgel ODS-80Ts QA
TSKgel ODS-120A	TSKgel ODS-120T	TSKgel OligoDNA-RP
TSKgel TMS-250		

Polymer-based:

TSKgel Octadecyl-2PW	TSKgel Octadecyl-4PW	TSKgel Octadecyl-NPR
TSKgel Phenyl-5PW RP		

Reversed Phase Tips:

- TSKgel reversed phase columns are offered in stainless steel hardware. Stainless steel (SS) frits are embedded in the body of the column endfittings. The nominal frit size for SS columns is engraved in the endfittings.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer.
- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column. In addition, guard cartridges, guardfilters, and packed guard columns are available for use with TSKgel reversed phase columns.
- Caution: The silica particles in TSKgel Super series columns have a relatively small pore volume, which results in shorter retention times than obtained on most other reversed phase columns. For instance, to achieve similar retention times as obtained on TSKgel ODS-100V, the percentage organic solvent in the mobile phase has to be reduced by about 5-10% on a TSKgel Super-ODS column.
- Optimizing results with the TSKgel Super series columns: TSKgel Super series columns can be used on a regular HPLC system if the dead volume is minimized, although optimal results are obtained with an HPLC system designed for 2 mm or smaller ID columns. The following recommendations are for 4.6 mm ID columns. Use proportionately lower values for 2 mm ID columns.

    1. A guard filter is highly recommended to reduce particulate contamination from the sample or system components.
    2. Keep sample volume less than 10 µL.
    3. To ensure minimal extra-column volume, keep tubing as short as possible (extra-column volume less than 5 µL between column and detector).
    4. Conventional 0.1 mm ID connecting tubing may be used (0.005”).
    5. The smallest detector time constant should be selected (if possible, less than 50 ms).
    6. The detector flow cell should be 2 µL or less for best results. A standard HPLC flow cell (10 µL) can be used as an alternative; however, it is recommended that the heating coil is removed.

- TSKgel reversed phase columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About Reversed Phase Chromatography

Reversed Phase Chromatography (RPLC or RPC) is the most efficient of all biomolecule separation techniques. It has been the technique of choice for the analysis of small molar mass compounds in both the pharmaceutical and chemical industries, as well as in biomedical research, since the late 1970s. More recently, RPC has become the accepted tool for the separation of peptides, proteins and other biopolymers, making it largely responsible for the widespread popularity of HPLC as a chromatographic technique.

The opposite of normal phase chromatography, RPC requires a non-polar stationary phase and a mobile phase that consists of a mixture of water and polar-solvent mobile phase. The so-called "hydrophobic effect" is the major driving force for retention in RPC. The hydrophobic effect is related to the non-polar surface area of the solute molecule, which varies as a function of mobile phase composition, while the strength of the hydrophobic bond is proportional to the decrease in molecular surface area when the solute associates with the carbon-based stationary phase. Mobile phase additives, such as trifluoroacetic acid, increase protein hydrophobicity by forming ion pairs that strongly adsorb to the stationary phase. Typically, the mobile phase consists of a mixture of water (buffer) and acetonitrile, methanol or, less common, THF, or 2-propanol. The biological molecules are eluted from the chromatographic support by a change in the polarity of the mobile phase.

Silica particles are most commonly used as the support, which then is derivatized with octadecylsilane (ODS). Polymer-based supports have been introduced as an alternative to silica-based reversed phase columns, particularly for analyzing basic compounds in their neutral state at high pH.

RPC columns can be applied to the analysis of a wide variety of compounds, ranging from neutral polar and non-polar solutes to acidic, basic, and amphoteric compounds. RPC is also an efficient technique for the analysis of derivatized amino acids, peptides and proteins, although protein structure is not always maintained due to the high concentration of organic solvent required for their elution.

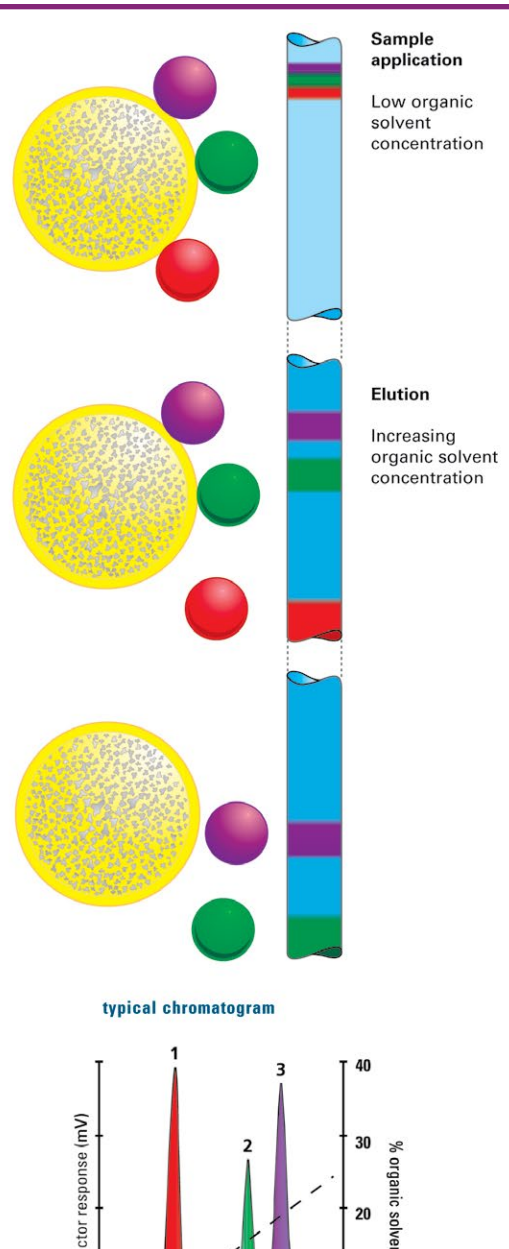
TSKgel Reversed Phase Chromatography Columns

Tosoh Bioscience offers 18 distinct Reversed Phase column types which are based on either silica or methacrylate particles, as discussed in Table 1.

Table 1: Overview of TSKgel RPC columns

Silica-based columns	Polymer-based columns
High purity Type B silica High efficiencies Excellent recoveries Low bleed for MS	Hydrophilic backbone to improve recovery and reduce secondary interactions. pH stable from 1 to 12. Compatibility with organic solvents eliminates swelling.
An excellent choice for analysis of small molecules and peptides. Grouped into 6 product families.	An excellent choice for large MW biomolecules (>1.0 × 10^4 Da) and for analyzing small MM compounds at high pH. Offered in 4 different chemistries.
• Protein C_4-300 • ODS-140HTP • ODS-100V and 100Z (10 nm) • Monomeric bonded silica (8 nm) • High efficiency (14 nm) • Specialty silica columns	• Octadecyl-2PW (12.5 nm) • Octadecyl-4PW (50 nm) • Phenyl-5PW RP (100 nm) • Octadecyl-NPR (nonporous)

Figure 1: Reversed Phase Chromatography



The silica-based TSKgel reversed phase product line consists of ten stationary phases designed for the analysis of low molar mass compounds, including pharmaceutical drugs, forensic compounds, derivatized amino acids, carbohydrates, steroids, lipids, and fatty acids, as well as two stationary phases with larger pore size designed for protein analysis.

TSKgel silica packings consist of spherical particles with uniform pore sizes of 8, 10, 12, 14, 25, or 30 nm bonded with a monomeric or polymeric layer of octadecyl, octyl, cyano, trimethylsilyl, or phenyl groups. Several of the stationary phases are subsequently derivatized with trimethylsilyl groups by a proprietary method that deactivates residual but accessible silanol groups.

Polymethacrylate-based reversed phase columns are available in a range of pore and particle sizes. Although often not as efficient as and less robust than silica-based RPC columns, key advantages of polymer-based columns are the fact that they are chemically stable from pH 2 to 12, which allows many basic compounds to be analyzed in their uncharged form, thus reducing secondary adsorption and improving peak shape and improving recovery for peptides and proteins due to reduced secondary interactions.

Tables 2 and 3 feature the properties and applications of each of the TSKgel silica-based and polymer-based reversed phase columns.

Table 2: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**End-capped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Protein C_4-300	C4 alkyl, polymeric	Yes	3	3	30	For recovery and resolution of large biomolecules, such as proteins
ODS-140HTP	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC applicable; high throughput separations; high resolution and short analysis time at moderate pressures
ODS-100V	C18 alkyl, monomeric	Yes	15	3, 5	10	Initial choice; general purpose column
ODS-100Z	C18 alkyl, monomeric	Yes	20	3, 5	10	Initial choice; general purpose column
ODS-120T	C18 alkyl, polymeric	Yes	22	5, 10	15	Specialty column for analysis of peptides, small proteins, and small molecular weight compounds
ODS-120A	C18 alkyl, polymeric	No	22	5, 10	15	Specialty column for analysis of polyaromatic hydrocarbons. Best choice for steric selectivity
ODS-80Ts	C18 alkyl, monomeric	Yes	15	5, 10	8	Low MW pharmaceuticals, bases, nucleosides and nucleotides. Ideal for strongly basic or charged compounds
ODS-80Ts QA	C18 alkyl, monomeric	Yes	15	5	8	Tighter specs than standard ODS-80Ts
ODS-80Tm	C18 alkyl, monomeric	Yes	15	5, 10	8	General purpose column for low MW pharmaceuticals, bases, nucleosides and nucleotides
Oligo-DNA RP	C18 alkyl, monomeric	No	10	5	25	For analysis and purification of oligonucleotides, RNA and DNA-fragments
Octyl-80Ts	C8 alkyl, monomeric	Yes	10	5	8	Ideal choice for highly hydrophobic small molecules; Reduced tailing when analyzing basic compounds

Table 2 Continued: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Super-ODS	C18 alkyl, polymeric	Yes	6	2.3	14	UHPLC-like resolution and speed with conventional HPLC systems; improved sensitivity; savings in time and solvent; less hydrophobic than C18; allows for rapid, high resolution separations of small proteins, pharmaceuticals, and aromatic compounds
Super-Octyl	C8 alkyl, polymeric	Yes	5	2.3	14	
Super-Phenyl	Phenyl alkyl, polymeric	Yes	3	2.3	14	
CN-80Ts	CN, monomeric	Yes	9	5	8	Polar peptides, amino acids, and other pharmaceutical and food & beverage products
TMS-250	C1 alkyl, monomeric	Yes	5	10	25	For recovery and resolution of large biomolecules, such as proteins

Table 3: Properties of TSKgel polymer-based RPC columns

Properties of Polymer-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Octadecyl-2PW	C18 alkyl, monomeric	-	-	5	12.5	Peptides up to 8,000 Da and small proteins
Octadecyl-4PW	C18 alkyl, monomeric	-	-	7, 13	50	Great for high pH separations of small molecules and proteins; Available in analytical and semi-preparative scale
Phenyl-5PW RP	Phenyl, monomeric	-	-	10, 13	100	Ideal for large, globular protein samples up to 1.0×10^6 Da; highly stable in low and high pH environments
Octadecyl-NPR	C18 alkyl, monomeric	-	-	2.5	nonporous	High efficiency separations and fast analysis of peptides and proteins with excellent pH stability