

RECOMMENDATIONS for USE, CLEANING and STORAGE of ZIRCONIA-BASED HPLC COLUMNS

ZirChrom®-Chiral(S)LEU

Thank you for purchasing this zirconia-based reversed phase high performance liquid chromatographic column from ZirChrom Separations. This product and/or its method of use is covered by one or more of the following patent(s): **US Patent No. 5,015,373, 5,108,597, 5,141,634, 5,205,929, 5,254,262, 7,897,798, Re: 34,910, 5,271,833, 5,346,619, 5,540,834, 6,846,410** and foreign equivalents. Additional patents are pending in the United States. We are sure you will be completely satisfied with its performance. In order to enjoy the tremendous benefits of its unique features compared to silica and polymer-based HPLC media, it is very important that you read the recommendations below. Please keep in mind that while this is a chiral column the substrate is zirconia, not silica, and the surface chemistry is completely different. The chiral selector for this column is (S)-3,5-dinitrobenzoyl-leucine. If at any time you have a question about this product we invite you to visit our web site (<http://www.zirchrom.com>) where you will find a complete list of over 70 technical articles in peer reviewed journals on zirconiabased HPLC. In addition, our staff is always eager to help you with any aspect of using this column (1-866-STABLE-1).

Use:

1. Upon receipt, we suggest you duplicate the results on the enclosed chromatogram. You should be able to achieve a plate count of at least **80,000 plates/meter** (*specification for 150 x 4.6 mm i.d. format*) for (R)-2,2,2-trifluoro-1-(9-anthryl)ethanol under the operating conditions given on the chromatogram. Be sure to inject roughly the same amount of material as indicated in the chromatogram.
2. For optimal column performance the mobile phase containing methanol or trifluoroacetic acid will significantly increase plate number, decrease retention time and change selectivity.
3. This column can be operated up to 50 °C without a loss in column efficiency.
4. This column is typically used for normal phase chiral separations. In an aqueous mobile phase the column is stable from pH 1 to 8. **Fluoride and phosphate** buffers should be avoided.
5. To maximize the life of this ultra-durable column, we recommend the following precautions regarding day-to-day operation of the column.
 - ✓ **Always use a guard column.**
 - ✓ Clean up samples before injection (either filtering to remove particulates or solid phase extraction techniques).
 - ✓ Use HPLC grade solvents and filter all solutions before use.
 - ✓ Minimize pressure surges.
 - ✓ Use an in-line filter (0.5 micron) in front of column to catch large particulates.
 - ✓ Flush all buffers and salts from column before storage.

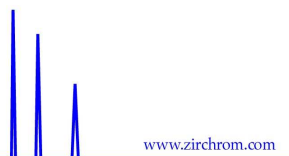
Cleaning/Regeneration:

Some organics may be adsorbed strongly to zirconia-based columns. To remove these from the zirconia surface, or to remove any substance that may have fouled the column, use the following two-step cleaning protocol:

1. Flush the column with methanol or isopropanol that contains 0.5% trifluoroacetic acid for 50 column volumes at ambient temperature. Follow the wash with 20 column volumes of isopropanol at ambient temperature.
2. Flush column with 50/50 isopropanol/hexane for 20 column volumes at ambient temperature. Follow organic wash with 20 column volumes of 98/2 hexane/isopropanol at ambient temperature.

Storage:

Flush the column according to the cleaning/regeneration protocol before long-term storage. The ZirChrom®-Chiral(S)LEU column should be stored in 98/2 hexane/isopropanol.





A complete list of chromatography products offered by ZirChrom Separations:

HPLC Columns

| Part # | Product Name | Chromatographic Mode |
|--------|------------------|--------------------------------------|
| DB01 | Diamondbond®-C18 | C18 Modified Carbon Reversed-phase |
| EZ01 | ZirChrom®-EZ | Deactivated Reversed-phase |
| MS01 | ZirChrom®-MS | Deactivated Reversed-phase for LC/MS |
| TI01 | Sachtopore®-RP | Reversed-phase (Titania) |
| TI02 | Sachtopore®-NP | Normal Phase (Titania) |
| ZR01 | ZirChrom®-CARB | Carbon Reversed-phase |
| ZR02 | ZirChrom®-PHASE | Normal Phase |
| ZR03 | ZirChrom®-PBD | Reversed-phase |
| ZR04 | ZirChrom®-WCX | Weak Cation-exchange |
| ZR05 | ZirChrom®-WAX | Weak Anion-exchange |
| ZR06 | ZirChrom®-SAX | Strong Anion-exchange |
| ZR07 | ZirChrom®-SHAX | Strong Hydrophilic |
| ZR08 | ZirChrom®-PEZ | Cation-exchange |
| ZR09 | ZirChrom®-PS | Reversed-phase |

Specialty Products

| Part # | Product Name | Chromatographic Mode |
|--------|---------------------------------|--------------------------------------|
| AB01 | Rhinophase-AB | Pseudo-Affinity Phase for Antibodies |
| BW01 | Advanced Buffer Wizard Software | 50 buffer systems (CD-ROM) |
| MK01 | Ion-exchange Method Kit #1 | SAX, SHAX, WAX |
| MK02 | Ion-exchange Method Kit #2 | SAX, WCX, PEZ |
| MK03 | Reversed-phase Method Kit #1 | PBD, CARB, DB01 |
| MK04 | Reversed-phase Method Kit #2 | EZ, CARB, PBD |
| NPZ | Nonporous Zirconia | 0.5, 1, 2, or 3 µm |
| ZRC01 | ZirChrom®-Chiral(S)LEU | Pirkle Type chiral phase |
| ZRC02 | ZirChrom®-Chiral(R)NESA | Pirkle Type chiral phase |
| ZRC03 | ZirChrom®-Chiral(S)NESA | Pirkle Type chiral phase |
| ZRC04 | ZirChrom®-Chiral(S)PG | Pirkle Type chiral phase |
| ZRC05 | ZirChrom®-Chiral(R)PG | Pirkle Type chiral phase |
| ZRC06 | ZirChrom®-CelluloZe | Polysaccharide chiral phase |

Note: All chromatography products are available in microbore, analytical, semi-preparative and preparative column formats.

