

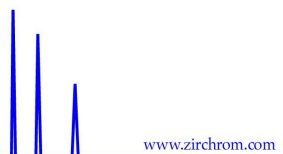
RECOMMENDATIONS for USE, CLEANING and STORAGE of ZIRCONIA-BASED HPLC COLUMNS

ZirChrom®-EZ

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, 8,137,548** 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 reversed-phase column the substrate is zirconia, not silica, and the non-polar stationary phase is cross-linked polybutadiene deposited in the pores and then the surface is deactivated, not alkylsilanes bonded to the surface. 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 70+ technical articles in peer reviewed journals on zirconia-based 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 **100,000 plates/meter** (*specification for 150 x 4.6 mm i.d format*) for toluene under the operating conditions given on the chromatogram. Be sure to inject roughly the same amount of material as indicated in the chromatogram.
2. This column can be used with any common organic modifier (acetonitrile, methanol, tetrahydrofuran, isopropanol). ZirChrom®-EZ is slightly less hydrophobic than common silica-based supports. For simple non-ionic compounds, we recommend that you use about **10% less organic modifier** to obtain roughly the same retention as you would on a typical C8 or C18 silicabased phase.
3. We very strongly advise that you use ZirChrom®-EZ at temperatures below **50°C**.
4. When running ionizable compounds on any stationary phase a buffer must be used (**see ZirChrom Buffer Wizard at www.zirchrom.com**). Our first choice for both acidic and basic analytes is 10-25 mM ammonium acetate at pH 5.0. However, this column is stable from pH 1-10** and a variety of buffers may be employed in this pH range. At present, we **DO NOT** recommend the use of high concentrations (>25 mM) of phosphate, fluoride, carboxylic acid (acetate, citrate, bicarbonate/carbonate) buffers, and salts.
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.**
 - ✓ ** ZirChrom®-EZ is stable up to pH 12 with the inclusion of special mobile phase additives (contact technical support for more information).
 - ✓ 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.
 - ✓ Always check the solubility of the buffer being used when mixing with organic mobile phases using an LC pump.



**Cleaning/Regeneration:**

To remove any substance that may have fouled the column, use the following three-step cleaning protocol:

****IMPORTANT**** - During these steps you should remove your detector from the flow path to protect it from aggressive cleaning conditions.

1. Flush column with a mixture of 20/80 acetonitrile/pH 10 ammonium hydroxide solution for 50 column volumes at ambient temperature. Follow base wash with 10 column volumes of water at ambient temperature.
2. Flush column with a mixture of 20/80 acetonitrile/0.1 M nitric acid for 50 column volumes at ambient temperature. Follow acid wash with 10 column volumes of water at ambient temperature.
3. Flush column with 100% organic solvent for 20 column volumes at ambient temperature. Methanol, acetonitrile, isopropanol, and tetrahydrofuran are all adequate solvents.

Storage:

The ZirChrom®-EZ column **should not be stored in phosphate buffer**. We strongly suggest flushing the column with 10 column volumes of 50/50 organic modifier/water prior to storage over night and with 10 column volumes of pH 10 ammonium hydroxide solution followed by 10 column volumes of 50/50 organic modifier/water before long-term storage.

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.

