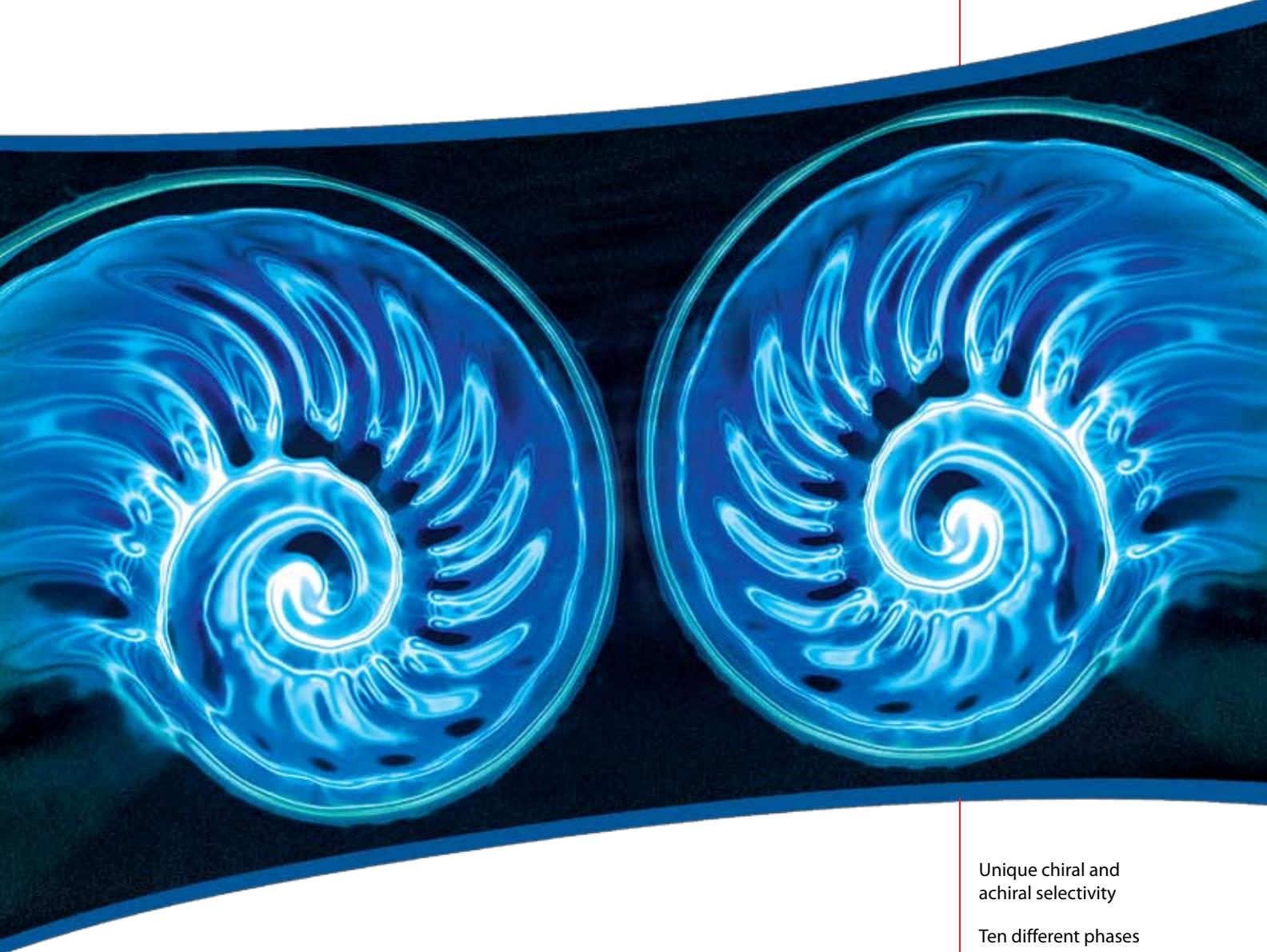


Astec CYCLOBOND

Bonded Cyclodextrin-based
Chiral HPLC Phases

 **SUPELCO**
Analytical



Unique chiral and
achiral selectivity

Ten different phases

RP, NP, LC-MS, and
SFC compatible

No solvent or additive
memory effects

Astec CYCLOBOND

The versatile and unique Astec CYCLOBOND™ CSPs (chiral stationary phases) are a family of derivatized and underderivatized β - and γ -cyclodextrins bonded to high-purity silica gel. Patented by Professor Daniel W. Armstrong and introduced to the market in 1984, they have found widespread use for isomer separations by HPLC, both chiral and achiral. Astec CYCLOBOND is complementary to other CSPs, including the polysaccharide-based CSPs, macrocyclic glycopeptide-based Astec CHIROBIOTIC® CSPs, and the amine copolymer-based Astec P-CAP™ and Astec P-CAP-DP.

Key Features and Application Areas

- Ideal for chiral analysis in the pharmaceutical industry, and for small analytes in chemical and environmental areas
- Routine chiral column method development screening protocols
- All chromatography modes: Reversed-phase, polar organic, normal phase, and SFC
- Complementary selectivity to other types of CSPs
- Highly compatible with LC-MS
- Scalable from analytical to preparative
- Covalently bonded for long column lifetime

What Makes Astec CYCLOBOND CSPs Unique?

CYCLOBOND CSPs offer unique chiral selectivity by way of multiple chiral mechanisms provided by the cyclodextrin cavity and the functional groups of the various derivatives. CYCLOBOND CSPs feature chemical stability for long lifetime, wide mobile phase choices, and high efficiency.

Astec CYCLOBOND I 2000 Series

- Native β -cyclodextrin and seven β -cyclodextrin derivatives bonded to high-purity silica gel
- Excellent chiral selectors for substituted phenyl, naphthyl, and biphenyl compounds

Astec CYCLOBOND II Series

- Native γ -cyclodextrin and the peracetylated derivative bonded to high-purity silica gel
- Excellent chiral selectors for multi-ring structures, such as those based on anthracene, chrysene, or pyrene

Table 1. The Astec CYCLOBOND CSP Family

Name	Cyclodextrin	Derivative (2- and 3-position hydroxyls)
Astec CYCLOBOND I 2000	Beta (β)	None (native)
Astec CYCLOBOND I 2000 AC	Beta (β)	Acetyl
Astec CYCLOBOND I 2000 DM	Beta (β)	Dimethyl
Astec CYCLOBOND I 2000 DMP	Beta (β)	3,5-Dimethylphenylcarbamate
Astec CYCLOBOND I 2000 DNP	Beta (β)	2,6-Dinitro-4-trifluoromethyl phenyl ether
Astec CYCLOBOND I 2000 SP	Beta (β)	S-Hydroxypropyl ether
Astec CYCLOBOND I 2000 RSP	Beta (β)	R,S-Hydroxypropyl ether
Astec CYCLOBOND I 2000 HP-RSP	Beta (β)	R,S-Hydroxypropyl ether
Astec CYCLOBOND II	Gamma (γ)	None (native)
Astec CYCLOBOND II AC	Gamma (γ)	Acetyl

TRADEMARKS: CHIROBIOTIC, CYCLOBOND, and P-CAP — Sigma-Aldrich Biotechnology, LP

Combining the Power of Cyclodextrin Architecture and Selective Surface Chemistry

What are Cyclodextrins?

Cyclodextrins are produced by partial degradation of starch, followed by the enzymatic coupling of glucose units into crystalline, homogeneous toroidal structures of different molecular size. The D(+) -glucose residues are bonded to each other through α -(1,4)glycosidic linkages. The chair configuration of glucose residues makes the toroid "bucket" narrower at one end (see **Figure 1**). Three highly-characterized cyclodextrins are alpha (α), beta (β), and gamma (γ) cyclodextrin, which contain six, seven, and eight glucose units, respectively (see **Table 2**). Because each glucose residue has five chiral centers, cyclodextrins are themselves chiral structures. For example, β -cyclodextrin has 35 chiral centers.

How Do Cyclodextrin-based CSPs Separate Enantiomers?

Both the architecture and chemistry of cyclodextrins contribute to enantiomer separations. The toroidal cyclodextrin structure has a hydrophilic exterior surface resulting from the 2-, 3-, and 6-position hydroxyl (OH) groups. The interior cyclodextrin cavity is composed of the glucose oxygens and methylene hydrogens, which gives it a non-polar (hydrophobic) character. Chemical interactions that lead to chiral separations occur on both the exterior and interior surfaces of the cyclodextrin toroid. The most

important consideration for retention and chiral recognition is proper fit of the analyte into the cyclodextrin cavity. This fit is a function of both molecular size and shape of the analyte relative to the cyclodextrin cavity. Thus, there are two basic mechanisms at play in chiral separations on cyclodextrins: those that occur on the inside cavity surface (inclusion complexing) and those that occur on the outside surface (surface interactions) of the cyclodextrin toroid.

Mechanism 1: Inclusion Complexing

The basis for many separations on cyclodextrin-based CSPs in the reversed-phase mode (mobile phases containing water with methanol or acetonitrile) is a phenomenon called inclusion complexing. If the analyte can fit into the cyclodextrin cavity and mobile phase conditions are favorable, the inclusion complexing mechanism can occur. It is because of inclusion complexing that reversed-phase is a very successful mode on CYCLOBOND CSPs. Three points of interaction are required for a chiral discrimination, and the inclusion complexing provides one of the three interactions.

The inclusion complexing mechanism is attributed to the attraction of the apolar molecule or segment of the molecule to the apolar cyclodextrin cavity, which is very sensitive to structural differences. When the analyte possesses an aromatic

Figure 1. Proposed Structure of Cyclodextrin Molecules

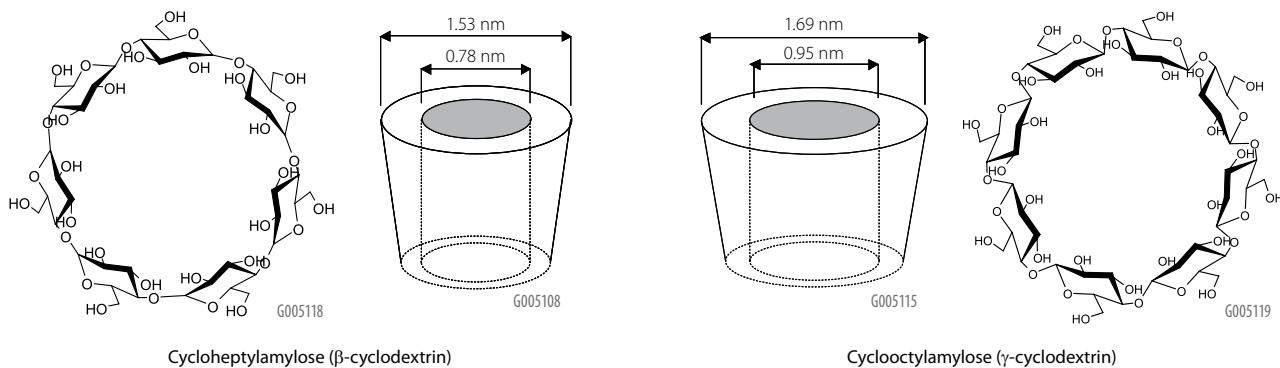


Table 2. Properties of Cyclodextrins

Cyclodextrin	Chemical Name	Glucose Units	Stereogenic Centers	Cavity Size (nm)
Alpha (α)*	Cyclohexylamylose	6	30	0.57
Beta (β)	Cycloheptylamylose	7	35	0.78
Gamma (γ)	Cyclooctylamylose	8	40	0.95

* α -CDs are currently not available as CSPs in the Astec line

group, the orientation in the cavity is selective due to the sharing of electrons between the aromatic methylene groups and the glucoside oxygens on the internal surface of the cyclodextrin toroid. The mechanism is completed by interaction of solute functional groups with the 2- and 3- position secondary hydroxyl groups of the cyclodextrin ring. A schematic of the inclusion mechanism is shown in Figure 2a. Linear or acyclic hydrocarbons occupy more random positions in the cavity. If a chiral separation is attempted in reversed-phase mode, it is therefore essential that the analyte have at least one aromatic ring or ring structure. The inclusion complexing mechanism also provides good separations of positional isomers. Inclusion complexing does not occur to the same extent in polar organic or normal phases modes because the non-polar attractions between analyte and the CD cavity are not favored.

Mechanism 2: Surface Interactions

In surface interactions, the chiral molecule lies across the external surface of the cyclodextrin toroid and interacts with the upper rim of the ring. Surface interactions dominate in polar organic (methanol or acetonitrile containing additives) and normal phase modes because in these modes analytes do not interact with the cyclodextrin cavity. This is for two reasons: First, when acetonitrile is present, it fully inserts into the cavity and blocks analytes from entering it. Second, when the mobile phase is totally non-aqueous, the non-polar interactions between analytes and the interior of the cyclodextrin cavity cannot occur. The surface interaction mechanism is depicted in Figure 2b.

Figure 2a: Inclusion Complexing Schematic

Representation of the inclusion complexing mechanism of an analyte into the cyclodextrin cavity. Subsequent interactions occur between the analyte and groups on the cyclodextrin surface. The analyte molecular size, shape and types of functional groups on it and the cyclodextrin contribute the enantioselectivity. Inclusion complexing occurs in reversed-phase mode.



Figure 2b: Surface Interaction Schematic

Representation of the surface interaction mechanism of an analyte with the cyclodextrin. These interactions dominate in polar organic and normal phase modes.



Functional Group Interaction

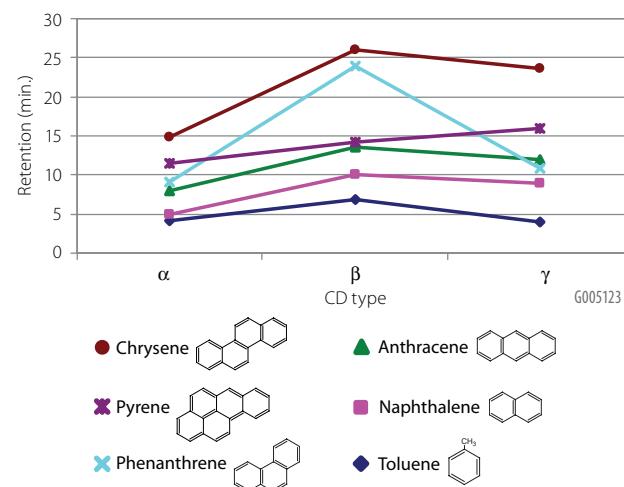
Certain analyte functional groups have a strong affinity for the cyclodextrin cavity. Other polar groups strongly hydrogen bond to the high-density hydroxyl surface of the native cyclodextrin. Derivatization of the cyclodextrin molecule at the 2- and 3-position hydroxyl groups affects selectivity and can be leveraged to alter the extent to which inclusion complexing occurs. For example, derivatized cyclodextrins, such as CYCLOBOND I 2000 DNP (dinitrophenyl) and CYCLOBOND I 2000 DMP (3,5-dimethylphenyl carbamate), provide additional interactions (π - π) as well as H-bonding. Halogens form strong inclusion complexes with these CSPs.

The affinity for the cyclodextrin cavity is influenced by functional groups, such as halogens, nitrates, sulfates, phosphates, and phenols on the analyte's aromatic rings. When these groups are present, inclusion complexing in reversed-phase mode is preferred. Protic substituents on the analyte, including carboxyls, carbonyls, amides, hydroxyls, and amines, generally provide surface interactions. Hydrogen bonding and dipole-dipole interactions also contribute to chiral selectivity.

Shape Selectivity

Figure 3 shows that for the cyclodextrin inclusion mechanism to occur, the molecular weight of a polyaromatic ring structure is not as critical as its footprint. The enantiomers of an analyte like norgestrel (a four-ring steroid structure) are better separated on the γ -cyclodextrin (CYCLOBOND II series, see Figure 12). The β -cyclodextrin (CYCLOBOND I 2000 series) is a better option for enantiomers of naphthalene-like structures or singly-substituted aromatic ring structures.

Figure 3. Molecular Size Selectivity on Cyclodextrin CSPs (same chemistry, different cavity size)



What Types of Enantiomers are Separated on Astec CYCLOBOND CSPs?

In general, substituted phenyl, naphthyl, and biphenyl rings can be separated on β -cyclodextrin-based CYCLOBOND I 2000 and its derivatives. Molecules with heterocyclic rings also often separate on these phases. Analytes with three to five rings, including steroids, are best separated on γ -cyclodextrin-based CYCLOBOND II and its derivatives. Enantiomers with halogens, nitrates, sulfates, phosphates, and hydroxyls on the analyte's aromatic rings generally separate well on CYCLOBOND CSPs. Also successfully resolved on CYCLOBOND are compounds with hydrogen-bonding functional groups off a ring, cis/trans and positional isomers (e.g. Figure 4), closely-related achiral molecules, and derivatized chiral amino acids.

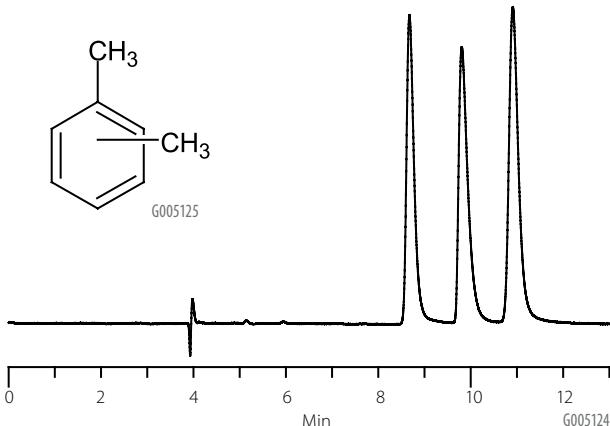
Mobile Phases for Astec CYCLOBOND CSPs

One of the important aspects of the CYCLOBOND family is that it operates in all mobile phase systems, permitting choice based on analyte solubility, detection method, or operator preference. It is interesting to note that temperature has dramatic effects on chiral selectivity on cyclodextrin phases.

- Reversed-phase: Water or buffers containing methanol or acetonitrile. Generally favors inclusion complexing.
- Normal phase & SFC: Hexane or CO_2 containing typical co-solvents (methanol, ethanol, IPA) and additives (TFA, TEA, DEA, etc.). Favors hydrogen bonding or π - π interaction on derivatized cyclodextrins (CYCLOBOND DMP and DNP only).
- Polar organic: Acetonitrile and/or methanol containing additives (acetic acid and triethylamine) that control hydrogen bonding or peak tailing. This mode enhances interactions with secondary hydroxyl groups across the cyclodextrin ring opening, as well as some functional groups found on derivatized cyclodextrins.

Figure 4. Positional Isomers (Xylenes) on Astec CYCLOBOND I 2000

column: CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 μm particles (20024AST)
mobile phase A: acetonitrile
mobile phase B: water
mobile phase ratio: 15:85 (A:B)
flow rate: 0.8 mL/min.
temp: 45 °C
det: UV, 230 nm
injection: 3 μL
sample: each compound, 0.1 mg/mL in acetonitrile:water (50:50)
elution order: m-, o-, p-xylene



Astec CYCLOBOND

Incorporating Astec CYCLOBOND into Your Chiral Column Screening Protocol

We recommend incorporating CYCLOBOND CSPs into your chiral column screening protocol. Their unique selectivity makes them complementary to other CSPs, and may provide the extra resolution needed to separate the target enantiomers.

Table 3 outlines our recommended method development screening protocol for CYCLOBOND columns in the different mobile phase systems.

Table 3. Astec CYCLOBOND Screening Protocol

Mobile Phase System	Starting Composition	Optimization
Reversed-phase (RP)	CH_3OH or CH_3CN /20 mM ammonium acetate, pH 5 (30:70)	Change % and type of organic modifier
Polar organic (POM)	$\text{CH}_3\text{CN}/\text{CH}_3\text{OH}$ /acetic acid/Triethylamine (95:5:0.1:0.1)	Use other polar organic solvents or blends. Test acid:base ratios from 1:4 to 4:1. Typical acid and base concentrations are 0.01 to 1%.
Normal phase (NP) (for CYCLOBOND DNP and DMP only)	Ethanol/Heptane (30:70)	Increase % of polar modifier. Change both solvents (e.g. IPA for ethanol, test any organic solvent)

Please request our Chiral Method Development poster (T409107) to see the complete Astec CHIROBIOTIC and CYCLOBOND column method development screening and optimization protocols.



Astec CYCLOBOND Native CSPs and Derivatives

The various CYCLOBOND phases are made by derivatization of the β - or γ -cyclodextrin molecule at the 2- or 3- position hydroxyl group. The 6-position OH is used to anchor the cyclodextrin to the silica surface. Selectivity is different among the CYCLOBOND family members. The phases described below are available in column formats for both analytical and preparative applications. Phases marked with an asterisk (*) are among the most popular CYCLOBOND phases, and are included in our Method Development Kit (20005AST).

Astec CYCLOBOND I 2000*

β -Cyclodextrin

CYCLOBOND I 2000 comprises β -cyclodextrin bonded by a patented process to produce a stable matrix with the cyclodextrin arranged to retain its most valuable property of forming inclusion complexes. This allows it to affect numerous chemical separations by selectively including into its cavity a wide variety of organic molecules. Non-inclusion-type separations are also possible with the polar organic mode for a wide variety of molecule types. Along with CYCLOBOND I 2000 HP-RSP, it is among the most popular of the Astec CYCLOBOND phases.

Astec CYCLOBOND I 2000 AC

β -Cyclodextrin, peracetylated

This is the peracetylated product of the native β -cyclodextrin. CYCLOBOND I 2000 AC is used primarily for aromatic alcohols or amines that are chiral on the alpha or beta carbon.

Astec CYCLOBOND I 2000 DM

β -Cyclodextrin, dimethylated

This is the dimethylated product of the native β -cyclodextrin. CYCLOBOND I 2000 DM separates a wide variety of structural and geometric isomers as well as a group of enantiomers not resolved on CYCLOBOND I 2000. This phase operates only in the reversed-phase mode by steric bulk as the main mechanism.

Figure 5. Ruelene (Cruiformate) on Astec CYCLOBOND I 2000

column: CYCLOBOND I 2000, 5 μ m particles (20024AST)
 mobile phase A: acetonitrile
 mobile phase B: acetic acid
 mobile phase C: Triethylamine
 mobile phase ratio: 100:0.3:0.2 (A:B:C)
 flow rate: 0.6 mL/min.
 temp.: 25 °C
 det.: UV, 254 nm
 injection: 5 μ L
 sample: Ruelene (cruiformate), 1 mg/mL in acetonitrile

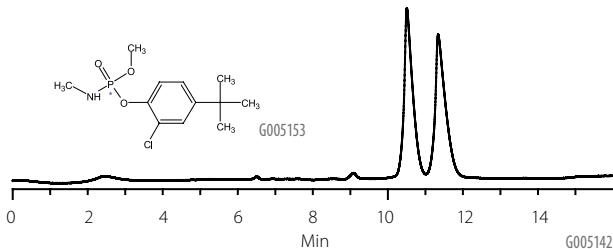


Figure 6. Norphenylephrine on Astec CYCLOBOND I 2000 AC

column: CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 μ m particles (20124AST)
 mobile phase A: methanol
 mobile phase B: 20 mM ammonium acetate, pH 5.0
 mobile phase ratio: 5:95 (A:B)
 flow rate: 0.5 mL/min.
 temp.: 25 °C
 det.: UV, 230 nm
 injection: 5 μ L
 sample: norphenylephrine, 1 mg/mL in acetonitrile:water (50:50)

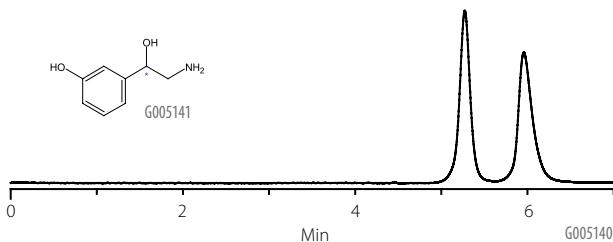


Figure 7. Coumachlor on Astec CYCLOBOND I 2000 DM

column: CYCLOBOND I 2000 DM, 25 cm x 4.6 mm I.D., 5 μ m particles (20924AST)
 mobile phase A: acetonitrile
 mobile phase B: 20 mM ammonium acetate, pH 2.9
 mobile phase ratio: 20:80 (A:B)
 flow rate: 0.8 mL/min.
 temp.: 25 °C
 det.: UV, 230 nm
 injection: 3 μ L
 sample: coumachlor, 1 mg/mL in acetonitrile:water (50:50)

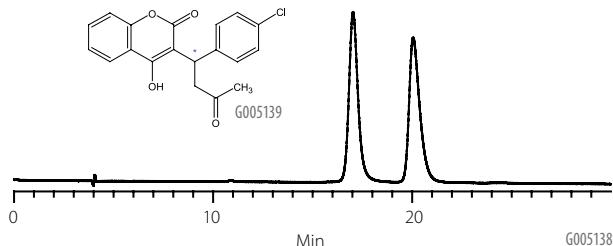
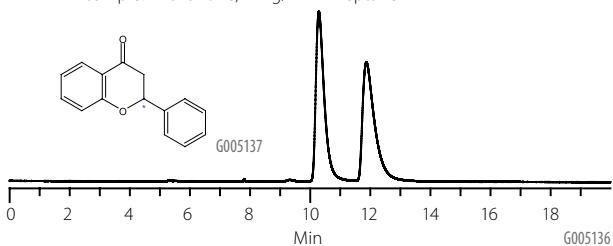
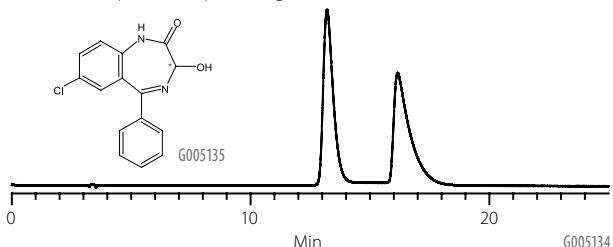


Figure 8. Flavanone on Astec CYCLOBOND I 2000 DMP

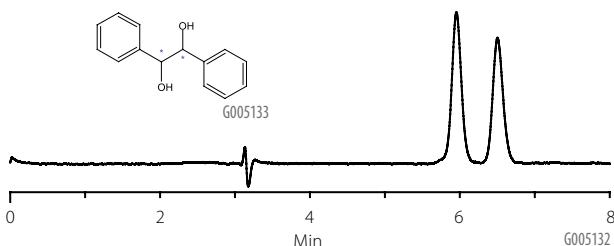
column: CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D.,
5 μ m particles (20724AST)
mobile phase A: isopropanol
mobile phase B: heptane
mobile phase ratio: 30:70 (A:B)
flow rate: 0.6 mL/min.
temp.: 25 °C
det.: UV, 254 nm
injection: 5 μ L
sample: flavanone, 1 mg/mL in heptane

**Figure 9. Oxazepam on Astec CYCLOBOND I 2000 DNP**

column: CYCLOBOND I 2000 DNP, 25 cm x 4.6 mm I.D.,
5 μ m particles (25024AST)
mobile phase A: acetonitrile
mobile phase B: 20 mM ammonium phosphate, pH 2.9
mobile phase ratio: 20:80 (A:B)
flow rate: 1 mL/min.
temp.: 5 °C
det.: UV, 254 nm
injection: 3 μ L
sample: oxazepam, 1 mg/mL in acetonitrile:water (50:50)

**Figure 10. Hydrobenzoin on Astec CYCLOBOND I 2000 RSP**

column: CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D.,
5 μ m particles (20324AST)
mobile phase A: acetonitrile
mobile phase B: 10 mM ammonium acetate, pH 4.0
mobile phase ratio: 25:75 (A:B)
flow rate: 1 mL/min.
temp.: 25 °C
det.: UV, 254 nm
injection: 5 μ L
sample: hydrobenzoin, 1 mg/mL in acetonitrile:water (50:50)

**Astec CYCLOBOND I 2000 DMP***

β -Cyclodextrin, 3,5-dimethylphenyl carbamate derivative

The reaction of the 3,5-dimethylphenyl isocyanate with some of the hydroxyl groups of β -cyclodextrin results in a π -basic phase similar in character to the naphthylethyl carbamate phases. The selectivity is greater for the CYCLOBOND I 2000 DMP when the analyte's chiral center is part of a ring structure or is on the alpha carbon. This phase can be operated in normal phase and polar organic phase modes, in addition to typical reversed-phase mode.

Astec CYCLOBOND I 2000 DNP*

β -Cyclodextrin, 2,6-Dinitro-4-trifluoromethyl phenyl ether derivative

This unique derivative has dinitrophenyl functionality bonded through an ether linkage to the β -CD. In this arrangement, a π -electron sharing system is established with analytes having π -systems in the stereogenic environment (e.g., aromatic rings, carbonyls). The π -acidity of this group is further enhanced with the introduction of the trifluoromethyl group in the aromatic ring. Use of the ether linkage to anchor this π -acidic dinitrophenyl ring results in a very stable system even under strong reversed-phase conditions. In a number of cases, selectivity was demonstrated only on CYCLOBOND I 2000 DNP; examples are ketorolac, oxazepam and compounds with multiple ring systems. While CYCLOBOND I 2000 DNP demonstrates selectivity in all three mobile phase conditions, reversed-phase conditions yield the greatest number of separations and highest selectivity. It has also been observed that buffers can have a dramatic affect on selectivity, especially when employing ammonium phosphate.

Astec CYCLOBOND I 2000 RSP

β -Cyclodextrin, R,S-hydroxypropyl ether derivative

A general-purpose chiral stationary phase that has the added property of separating non-aromatic structures such as t-Boc amino acids, for which it is a standard methodology.



Astec CYCLOBOND I 2000 HP-RSP*

High Performance β -Cyclodextrin, *R,S*-hydroxypropyl ether derivative

In the design of this new chemistry, it was an objective to create a very stable and reproducible phase with shorter retention times, while maintaining or improving selectivity compared with CYCLOBOND I 2000 RSP. After an extensive evaluation of this new chemistry, that goal was attained, as well as a dramatic improvement in a number of separations. CYCLOBOND I 2000 HP-RSP separates by extended H-bonding capability, offers broad chiral selectivity for chiral screening, and is most beneficial for basic and neutral compounds. Along with CYCLOBOND I 2000, it is among the most popular of the Astec CYCLOBOND phases.

Astec CYCLOBOND I 2000 SP

β -Cyclodextrin, *S*-hydroxypropyl ether derivative

In this phase, the hydroxyl groups on the surface of the β -cyclodextrin are reacted with (S)-propylene oxide. This has the effect of extending hydrogen-bonding capabilities to accommodate greater distances of the chiral center from an aromatic ring structure.

Astec CYCLOBOND II

Native γ -Cyclodextrin

CYCLOBOND II is γ -cyclodextrin bonded to silica. An excellent chiral selector for multi-ring structures, it is useful for isomeric compounds based on anthracene, chrysene and pyrene-type ring structures. CYCLOBOND II offers good selectivity and stability, and is applicable to the polar organic and reversed-phase modes. Applications include steroids, porphyrins, and FMOC amino acids.

Astec CYCLOBOND II AC

γ -Cyclodextrin, acetylated

CYCLOBOND II AC columns utilize γ -cyclodextrin with the hydroxyl groups in the 2 and 3 position acetylated. As a result, the mouth of the cavity has a hydrogen acceptor site suitable to interact with a hydrogen donor, such as an amine attached to at least two fused rings or larger. An example would be 1- or 2- substituted naphthylethylamine. Applications include steroids and sterols, depending on where the hydroxyl groups are positioned.

* Phases included in Method Development Kit (20005AST).

Figure 11. Miconazole on Astec CYCLOBOND I 2000 HP-RSP

column: CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 μ m particles (24024AST)
 mobile phase A: acetonitrile
 mobile phase B: 20 mM ammonium acetate, pH 4.0
 mobile phase ratio: 25:75 (A:B)
 flow rate: 0.6 mL/min.
 temp.: 25 °C
 det.: UV, 230 nm
 injection: 5 μ L
 sample: miconazole, 1 mg/mL in acetonitrile:water (50:50)

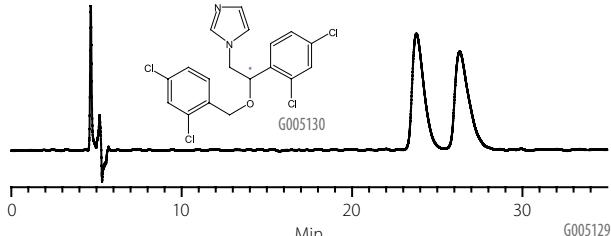
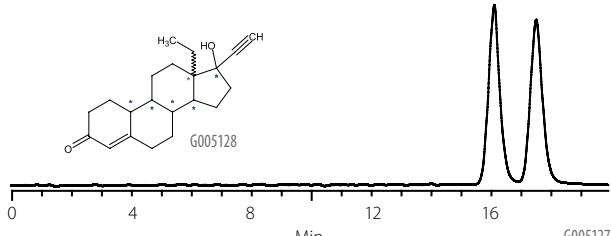


Figure 12. Norgestrel on Astec CYCLOBOND II

column: CYCLOBOND II, 25 cm x 4.6 mm I.D., 5 μ m particles (41020AST)
 mobile phase A: water
 mobile phase B: acetonitrile
 mobile phase ratio: 70:30 (A:B)
 flow rate: 0.8 mL/min.
 temp.: 22 °C
 det.: UV, 254 nm
 injection: 1 μ L
 sample: norgestrel, 1 mg/mL in methanol



Method Development on CYCLOBOND Columns

Astec CYCLOBOND columns should be part of any chiral HPLC column screening protocols.

With that in mind, we have developed column screening and optimization protocols and published them in a convenient wall chart format (T409107).

To request a copy, please contact your local Sigma-Aldrich/Supelco office or visit our website:

sigma-aldrich.com/chiral



Chiral HPLC Method Development Screen on Astec CHIROBIOTIC™ & CYCLOBOND™

Introduction

These screening protocols are used by our Chiral Applications Laboratory personnel to provide a rapid determination of the most suitable CHIROBIOTIC or CYCLOBOND column and mobile phase combination for an enantioseparation.

Column Installation & Conditioning

- CHIROBIOTIC columns are aligned in methanol. Fluid columns (25 cm x 4.6 mm I.D. columns) are aligned in 50 mM ammonium acetate (50:50) to reduce their ionic character.
- For reversed-phase mode, flush with 10 column volumes of methanol, then 10 column volumes of 50 mM ammonium acetate.
- For both columns, flush with 10 column volumes of methanol, then 10 column volumes of 50 mM ammonium acetate.
- If columns will be used in normal phase mode, flush with 10 column volumes of 50 mM ammonium acetate.
- If columns will be used in normal phase mode, flush with 10 column volumes of 50 mM ammonium acetate.
- For reversed-phase mode, flush with 10 column volumes of operating mobile phase.
- If columns will be used in normal phase mode, flush with 10 column volumes of operating mobile phase.
- Once columns are aligned, flush with 10 column volumes of 50 mM ammonium acetate initially, but once baseline is achieved it is recommended to flush with 10 column volumes of 50 mM ammonium acetate.

* Approx. 40 mLs, pH 7.0 ± 0.1, 40 °C, 1 mL/min, 25 cm x 4.6 mm I.D. columns.

Operating Parameters & Compatibility

- CHIROBIOTIC and CYCLOBOND columns are chemically bonded and compatible with all conventional HPLC solvents and buffers, including organic solvents. Use the following parameters to avoid a pH outside the recommended range.

• pH Range: pH 3 to 7
• Temperature: 10 to 40 °C
• Pressure: < 3500 psi (240 bar)
• Column: 25 cm x 4.6 mm I.D. (0.5 µm)

* Temperatures up to 30 °C are possible, but column lifetime may be compromised. Higher temperatures may reduce column lifetime. It is recommended to use the minimum temperature required to achieve the desired resolution. Higher temperatures may increase column efficiency, but may also increase column back pressure and reduce column lifetime.

Optimization

Mobile Phase System

- Test acid/base ratios from 1:4 to 1:10 after retention and selectivity.
- Typical acid and base concentrations are 0.03 to 1%.
- Change % organic solvent.
- Replace acid and base with a volatile salt, concentration 0.005 to 0.5% (can be tested using a concentration gradient). Try different salts.
- Adjust pH.

Reversed-phase (RP)

- Change % and type of organic modifier.
- Adjust pH, buffer type, ionic strength.
- Use different salt concentrations (e.g. 50 mM ammonium acetate).

Polar organic (POM)

- Use different salt concentrations (e.g. 50 mM ammonium acetate).
- CHC6H5CO (CHC6H5CO) Test acid/base ratios from 1:4 to 1:10 after retention and selectivity.
- Typical acid and base concentrations are 0.03 to 1%.
- Change % organic solvent.
- Change both solvents (e.g. IPA for ethanol, test any organic solvent).

Normal phase (NP)

- Change % organic solvent.
- Change acid/base ratio.
- Lower pH.

Optimization Based on Analyte Type

Mode	CHIROBIOTIC		CYCLOBOND	
	Polar Ionic	Reversed-Phase	Polar Organic	Reversed-Phase
Acidic Compounds	More base (TFA or other), Lower pH (3-4)	Higher pH (8-9)	More acid (TFA or other), Lower pH (3-4)	Lower pH (3-4)
Basic Compounds	More acid (TFA or other), Lower pH (3-4)	More base (TEA or other), Lower pH (3-4)	Evaluate best option	Lower pH (3-4)
Neutral Compounds	Acid or base (not needed), use 100% CH3OH	Lower pH	No change in ionic ratio needed	Lower pH (3-4)

CHIROBIOTIC Screening Protocol

Mobile Phase System

- Polar Ionic (PI): CH3OH:water:TEA (10.0:1:0.1 v/v/v)
- Reversed-phase (RP): CH3OH:CHC6H5CO:0.5 mM ammonium acetate, pH 5 (30:70)
- Polar organic (POM): Ethanol
- Normal phase (NP): Ethanol

Conditions (for 25 cm x 4.6 mm I.D. columns): Flow rate: 1.0 mL/min, Sample: 2-5 µL of 1.2 mg/mL in CH3OH or CH2Cl2

Notes:

- Allow 10 column volumes for equilibration in new mobile phase.
- Allow 10 column volumes for equilibration in new mobile phase if there is no elution after 30 minutes. Change pH, a volatile salt, or both solvents.
- Replace organic (PI), POM, or NP with TEA, but it will be different.

CYCLOBOND Screening Protocol

Mobile Phase System

- Polar Ionic (PI): CH3OH:20 mM ammonium acetate, pH 5 (30:70)
- Reversed-phase (RP): CH3OH:20 mM ammonium acetate, pH 5 (30:70)
- Polar organic (POM): CHC6H5CO:CHC6H5CO:0.5 mM ammonium acetate, pH 5 (30:70)
- Normal phase (NP): Ethanol

Conditions (for 25 cm x 4.6 mm I.D. columns): Flow rate: 1.0 mL/min, Sample: 2-5 µL of 1.2 mg/mL in CH3OH or CH2Cl2

Notes:

- Allow 10 column volumes for equilibration in new mobile phase.
- Allow 10 column volumes for equilibration in new mobile phase if there is no elution after 30 minutes. Change pH, a volatile salt, or both solvents.
- CH3OH and CH2Cl2 can show large differences in CYCLOBOND in reversed-phase mode.

LC-MS Optimization

CHIROBIOTIC Use volatile salts in POM (e.g. ammonium acetate, ammonium formate, ammonium TEA) in POM, lower concentration by 50-75%
CYCLOBOND Use volatile salts in POM (e.g. ammonium acetate, ammonium formate) in POM, lower concentration by 50-75%
Both columns Use ammonium acetate or ammonium formate in POM

Column Reconditioning & Storage

CHIROBIOTIC Columns

- Recondition with 10 column volumes of methanol, then 10 column volumes of 50 mM ammonium acetate (50:50) to restore their ionic character.
- Recondition with 10 column volumes of methanol or acetone before use, or if only a single, sharp peak is observed.
- CH3OH and CH2Cl2 can show large differences in CYCLOBOND in reversed-phase mode.

CYCLOBOND Columns

- Conditioning: Fluid columns with 10 column volumes of methanol, then 10 column volumes of 50 mM ammonium acetate (50:50) to restore their ionic character.
- Conditioning: Fluid columns with 10 column volumes of methanol, then 10 column volumes of 50 mM ammonium acetate (50:50) to restore their ionic character.
- Storage: Methanol or acetone are suitable for short term storage. Acetone is recommended for longer term storage (>24 hours) isopropanol is recommended.

Resting Columns

CHIROBIOTIC Columns

- To ensure the selectivity performance of CHIROBIOTIC columns, periodically wash with 50% methanol/50% methanol (4000:1) column in 100% methanol mobile phase.

CYCLOBOND Columns

- Mobile phase: 50% IPA report, consult our web site or call Technical Services for the test procedure.

Chiral Services

Chiral column screening, method optimization, and small-scale enantioseparation services are available from Supelco/Sigma-Aldrich. For more information, visit our website at sigma-aldrich.com/chiral, or contact our Technical Services Department.

U.S. Lab:
Phone: 800-359-3041 / 814-359-3041
Fax: 814-359-3048
Email: astech@supelco.com

Europe:
Phone: +44-12-345-2345
Fax: +44-12-345-2348
Email: astech@supelco.com

Supelco Chiral Services

Our Chiral Services Laboratory is distinct in offering HPLC column screening in normal phase and LC-MS-compatible reversed-phase, polar ionic and polar organic separation modes, using Astec Cellulose, CHIROBIOTIC, CYCLOBOND, P-CAP and other CSPs and modes as dictated by sample solubility and specific customer requirements. Consult Supelco to obtain a quotation for our expert services for chiral column screening (HPLC and GC), method optimization, and isolation of up to 10 grams of purified enantiomer. Purifications above 10 grams to production scale are available through our SAFC partners worldwide. The complete listing of our chiral HPLC and GC columns and chiral services can be found at sigma-aldrich.com/chiral our corporate chiral web portal. Here you can view our other products for chiral chemistry, like chiral catalysts, building blocks, mobile phase additives, derivatization reagents, and more.

P001331

TECHNICAL SERVICE: 800-359-3041 (US and Canada only) / 814-359-3041

 **SUPELCO**
Analytical

9

Astec CYCLOBOND

Astec CYCLOBOND Columns

Other dimensions are available, please visit our web site or contact techservice@sial.com

Cat. No.	Particle Size (µm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND I 2000			
20019AST	5	15	2.1
20020AST	5	25	2.1
20023AST	5	15	4.6
20024AST	5	25	4.6
20034AST	5	25	10
20044AST	5	25	21.2
Guards			
21010AST	5	2	1
21100AST*	5	2	4
Astec CYCLOBOND I 2000 RSP			
20319AST	5	15	2.1
20320AST	5	25	2.1
20323AST	5	15	4.6
20324AST	5	25	4.6
20334AST	5	25	10
20344AST	5	25	21.2
Guards			
21013AST	5	2	1
21103AST*	5	2	4
Astec CYCLOBOND I 2000 DMP			
20719AST	5	15	2.1
20720AST	5	25	2.1
20723AST	5	15	4.6
20724AST	5	25	4.6
20734AST	5	25	10
20744AST	5	25	21.2
Guards			
21017AST	5	2	1
21107AST*	5	2	4
Astec CYCLOBOND I 2000 SP			
20219AST	5	15	2.1
20220AST	5	25	2.1
20223AST	5	15	4.6
20224AST	5	25	4.6
20234AST	5	25	10
20244AST	5	25	21.2
Guards			
21012AST	5	2	1
21102AST*	5	2	4

Cat. No.	Particle Size (µm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND I 2000 AC			
20119AST	5	15	2.1
20120AST	5	25	2.1
20123AST	5	15	4.6
20124AST	5	25	4.6
20134AST	5	25	10
20144AST	5	25	21.2
Guards			
21011AST	5	2	1
21101AST*	5	2	4
Astec CYCLOBOND I 2000 HP-RSP			
24019AST	5	15	2.1
24020AST	5	25	2.1
24023AST	5	15	4.6
24024AST	5	25	4.6
24034AST	5	25	10
24044AST	5	25	21.2
Guards			
24101AST	5	2	1
24100AST*	5	2	4
Astec CYCLOBOND I 2000 DNP			
25019AST	5	15	2.1
25020AST	5	25	2.1
25023AST	5	15	4.6
25024AST	5	25	4.6
25034AST	5	25	10
25044AST	5	25	21.2
Guards			
25101AST	5	2	1
25100AST*	5	2	4
Astec CYCLOBOND I 2000 DM			
20919AST	5	15	2.1
20920AST	5	25	2.1
20923AST	5	15	4.6
20924AST	5	25	4.6
20934AST	5	25	10
20944AST	5	25	21.2
Guards			
21019AST	5	2	1
21109AST*	5	2	4

Guard Column Holders



P001316

Guard Holders for 4 mm I.D. cartridges (holder not required for 1 mm I.D. guards)

Cat. No.	Description
21150AST	Guard Column Holder

Cat. No.	Particle Size (µm)	Length (cm)	I.D. (mm)
Astec CYCLOBOND II			
46019AST	5	15	2.1
41021AST	5	25	2.1
46023AST	5	15	4.6
41020AST	5	25	4.6
40025AST	5	25	10
40028AST	5	25	21.2
Guards			
41001AST	5	2	1
42120AST*	5	2	4
Astec CYCLOBOND IIAC			
47019AST	5	15	2.1
41024AST	5	25	2.1
47023AST	5	15	4.6
41022AST	5	25	4.6
40420AST	5	25	10
40422AST	5	25	21.2
Guards			
41002AST	5	2	1
42121AST*	5	2	4

* Requires guard column holder (21150AST)..

Astec CYCLOBOND

Astec CYCLOBOND Method Development Kit

The kit contains one 25 cm x 4.6 mm I.D. column of each of our four most popular Astec CYCLOBOND phases: CYCLOBOND I 2000, CYCLOBOND I 2000 HP-RSP, CYCLOBOND I 2000 DMP, and CYCLOBOND I 2000 DNP. Because the price of the kit is below that of the columns sold separately, it is an economical means to augment your chiral column library.

Cat. No.	Description
20005AST	Astec CYCLOBOND Method Development Kit

Sigma-Aldrich® Worldwide Offices

Argentina

Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia

Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria

Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium

Free Tel: 0800 14747
Free Fax: 0800 14745
Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil

Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada

Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile

Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

China

Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic

Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark

Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland

Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France

Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany

Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1160

Hungary

Ingyenes telefonszám: 06 80 355 355
Ingyenes fax szám: 06 80 344 344
Tel: (+36) 1 235 9063
Fax: (+36) 1 269 6470

India

Telephone

Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 2570 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8003
Fax
Bangalore: (+91) 80 6621 9550
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 4087 2364
Hyderabad: (+91) 40 4015 5488
Kolkata: (+91) 33 4013 8000

Ireland

Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: (+353) 402 20370
Fax: (+353) 402 20375

Israel

Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4100
Fax: (+972) 8 948 4200

Italy

Free Tel: 800 827 018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan

Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea

Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Malaysia

Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico

Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands

Free Tel: 0800 022 9088
Free Fax: 0800 022 9089
Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand

Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway

Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland

Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal

Free Tel: 800 202 180
Free Fax: 800 202 178
Tel: (+351) 21 924 2555
Fax: (+351) 21 924 2610

Russia

Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore

Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia

Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa

Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain

Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden

Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland

Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2828
Fax: (+41) 81 755 2815

United Kingdom

Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

United States

Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam

Tel: (+84) 3516 2810
Fax: (+84) 6258 4238

Internet

sigma-aldrich.com



Accelerating Customers'
Success through Innovation and
Leadership in Life Science,
High Technology and Service

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries **SAFC®** (800) 244-1173
Safety-related Information sigma-aldrich.com/safetycenter

World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

©2010 Sigma-Aldrich Co. All rights reserved. SIGMA, SAFC, SIGMA-ALDRICH, ALDRICH, FLUKA, and SUPELCO are trademarks belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, LP. Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.

MRX
T410091