

ChiroSolve Inc.

616 Stendhal Ln., Cupertino, CA 95014, USA
 Telephone: (408) 834-8597; Fax: (408) 351-7900

Website: <http://www.chirosolve.com> | Technical/Customer Support: info@chirosolve.com | Sales support: sales@chirosolve.com

ChiroSolv® EnantioPrep Kits Deliver Pure Enantiomers

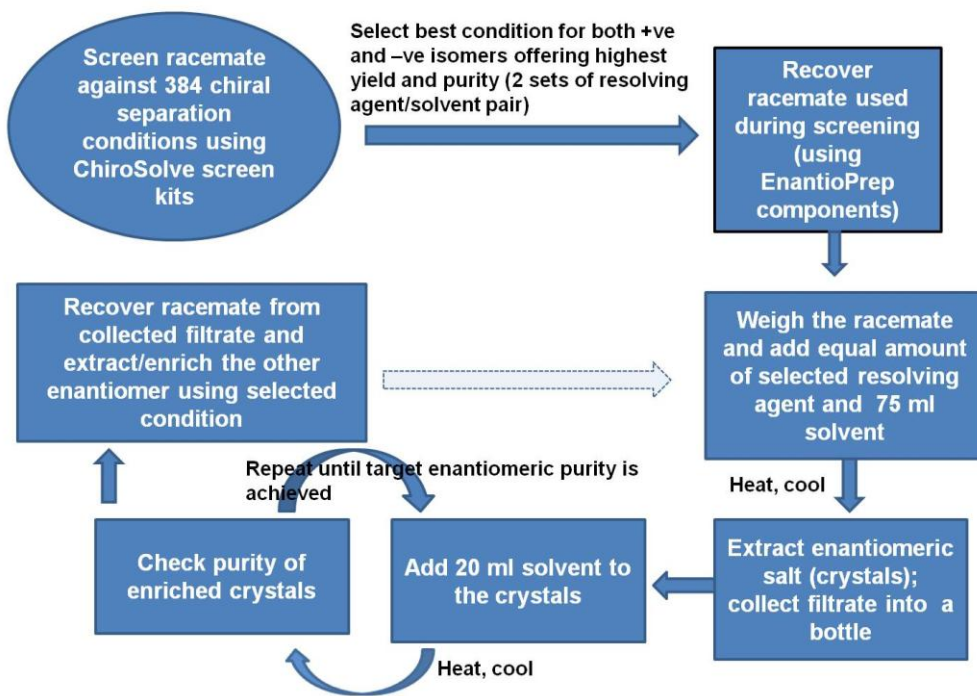
ChiroSolve, Inc. is pleased to introduce a ready-to-use kit solution named EnantioPrep that defines scalable, optimized chiral resolution method and that delivers pure enantiomer (**both +ve and -ve**) within matter of days.

EnantioPrep is designed to obviate the obstacles analytical chemists face during the elaborate process of diastereomeric crystallization. It offers cost efficient well-established method to identify a comprehensive set of chiral separation options, deliver pure enantiomer and define a scalable chiral separation. What sets EnantioPrep apart from other method development tools is the inclusion of ChiroSolv® Screening Kits that allow for 384 unique reagent combinations to identify optimum separation conditions. It is an extremely time and resource efficient approach to chiral resolution that allows analytical chemists to skip over the hard part of finding an optimal or scalable method to achieving an enantiopure product.

EnantioPrep allows scientists to:

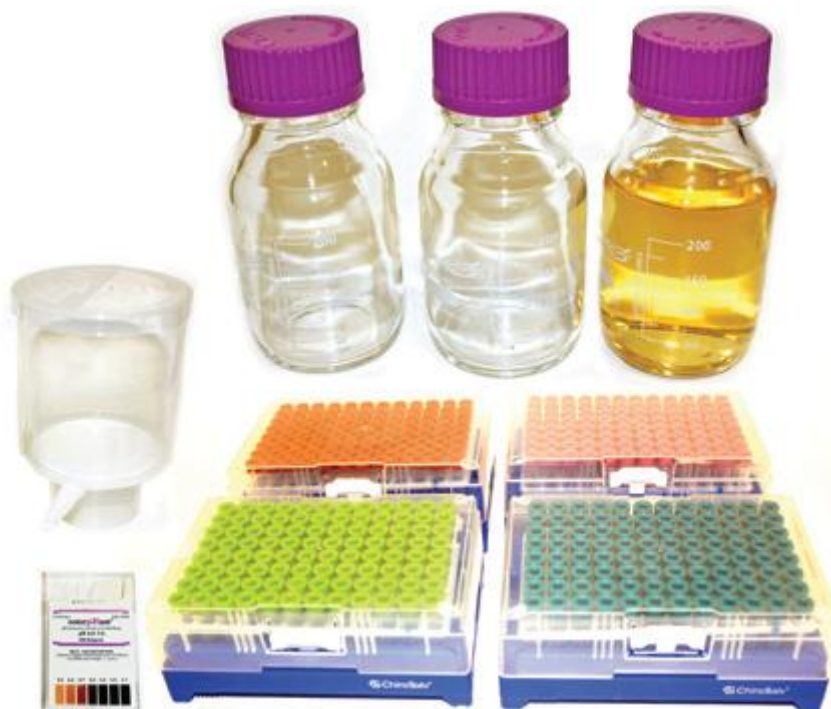
- Quickly screen a racemate against a comprehensive list of 384 resolving agent/solvent combinations to identify full scope of commercially viable resolution conditions
- Recover the used racemate and proceed to separate and purify target enantiomer (+ve or -ve) using selected separation condition until target purity is achieved
- If the second enantiomer is also needed, recover the racemate from collected filtrate using spare recovery solution and repeat the above purification
- Define a step-by-step resolution method that can be scaled up to kilo quantities

ENANTIOPREP



This solution can be used for racemic acids, bases, alcohols, amino acids, aldehydes, and ketones.

EnantioPrep includes:



- Screen kit (has 4 reagent plates and 4 solvent plates)
- 2 bottles of “Recovery Solution” (one for each +ve and –ve enantiomer) for acid or base racemate that recovers up to 90% racemate used during screening
- Self-contained Purification Package that allows repeated re-crystallization of pure enantiomer through simple steps to give both +ve and –ve enantiomer and to define consistent separation method

Individual reagent plates in the screen kit are in disposable, ready-to-use, 96-vial, high-throughput format that allows the entire analysis to be performed inside the plate without having to take out individual vials. The plates and the racks are made of polypropylene material that can withstand extreme temperatures (–20 to 120 °C), allowing the entire plate to be placed in a hot-water or ice bath without damage. Each Screen Kit offers unique 384 combinations of resolving agent/solvent combinations, so that comprehensive selection of separation conditions can be identified early on to help rest of the research. Resolving agents are chosen with manufacturing use in mind; they are relatively inexpensive and recoverable in high yield after the resolution is complete. The high-throughput format allows scientists to explore the separation conditions in parallel and get results within 24 hours, which may otherwise take over 2 months.

Once the screening work is accomplished and ideal separation conditions are identified for both +ve and –ve enantiomer, most of the racemate used during screening can be recovered back. When the recovery solution is added to the diastereomeric salts of the racemate and the resolving agents, it breaks the diastereomeric salt into neutral racemate that goes in the organic layer; while the resolving agents stay in the aqueous layer. Separating these two layers using a separatory funnel and removing the residual solvents from the organic layer gives back the original racemate.

During purification, a filter funnel with vacuum adapter as well as additional disposable filters is provided to enable quick and easy re-crystallization steps. We also provide “Filtrate Collection Bottle”, “Enantiomer Collection Bottle”, and pH paper.

After recovering the racemate from screening kits (using the Recovery Solution) and removing the residual solvents from it, scientist selects the ideal separation condition for the target enantiomer (using Screening results) and adds the

associated resolving agent and solvent combination to be recovered racemate and develops enantiomeric salt (as done during screening) of the desired enantiomer, This is salt is further purified using the above mentioned filter funnel and other components, until the required purity is achieved. Finally the enantiomer is extracted from the diastereomeric salt. In our experience, if you use 5 gm of racemate during purification, depending on the number of re-crystallization steps needed, you can get up to 2 gm of pure enantiomer. The entire purification typically requires up to 8 hours of hands-on work giving results within 1 week.

Types of Screening Kits

Acid

It includes 4 acid reagent plates each with 96 vials that contain distinct group of chirally pure acids and 4 solvent plates. They are used to resolve racemic bases and amino acids (some pre-processing is required for amino acids). Examples of resolving agents in the acid kit are:

- (–)-Camphoric acid
- (+)- and (–)-Camphorsulphonic acid
- (+)- and (–)-Dibenzoyltartaric acid
- (–)-Malic acid
- (+)- and (–)-Mandelic acid
- (+)-Lactic acid
- (+)- and (–)-Tartaric acid

Base

It includes 4 base reagent plates each with 96 vials that contain distinct group of chirally pure bases and 4 solvent plates. They are used to resolve racemic acids, alcohols, aldehydes, and ketones (some pre-processing is required for the latter three). Examples of resolving agents in the base kits are:

- (–)-2-Aminobutanol
- (–)-Brucine
- (–)-Cinchonidine
- (+)-Cinchonine
- (+)-Dehydroabietylamine
- (+)- and (–)-Methylbenzylamine
- (+)-Quinidine
- (–)-Quinine

Case Studies with ChiroSolv® EnantioPrep solution

ChiroSolv EnantioPrep kits were used against two sample racemates:

- **N-benzyl-1-(4-methylphenyl)propan-2-amine** (racemate had 70% S and 30% R isomer). Goal was to get over 90% purity for R isomer. This racemate was resolved by Di-tolyltararic (+) acid in 100% IPA. After two re-crystallizations we were able to obtain 30% S and 70% R. Additional 2 re-crystallizations yielded > 90% enrichment in R. The yield was over 90% of theoretical value.
- **N-Benzyl-(4-benzylphenyl)propane-2-amine** (racemate had 50% S and 50% R). Goal was to get over 90% purity for R isomer. This racemate was resolved by S-Acetylmandelic acid (+) in 90% IPA. After two re-crystallizations, we were able to obtain 80% R and 20% S, with yield over 80% of theoretical value. We expect that one more re-crystallization will yield over 90% purity in R.

Racemate	Desired isomer purity	Starting material	Amount recovered	# re-crystallization	Amount of pure enantiomer
N-Benzyl-(4-methylphenyl)propane-2-amine	R isomer, >90% purity	1.046 gm	0.996 gm (95%)	4	380 mg
N-benzyl-1-(4-	R isomer, >90%	1.931 gm	1.738 gm (90%)	3	700 mg

benzylphenyl)propane-2-amine	purity				
------------------------------	--------	--	--	--	--

How Do EnantioPrep Kits Work?

ChiroSolv Kits use the method of diastereomeric crystallization, a process that chemically separates enantiomers in a racemic mixture by complexation with an enantiopure acid or base, resulting in a mixture of diastereomeric salts. These salts have different chemical and physical properties, which allow their separation. This well established technology competes favorably with newer techniques such as asymmetric synthesis, biocatalysis, enzyme resolution, kinetic resolution, or chiral chromatography.

One major advantage of diastereomeric crystallization is that the procedure scales up easily for manufacturing purposes. Today around 65% of chiral products are made using this technique.

Industry examples:

- Paroxetine is a selective serotonin reuptake inhibitor developed by Novo Nordisk's subsidiary, Ferrosan, and licensed to GlaxoSmithKline. It is sold as the hydrochloride salt under the name Paxil® for the treatment of depression, anxiety, and obsessive–compulsive disorder. It is prepared by resolution using (–)-di-*p*-toluoyl-*l*-tartaric acid.
- Setraline is an antidepressant that inhibits the uptake of serotonin in the central nervous system and is sold by Pfizer under the name Zoloft®. Diastereomeric crystallization of the racemate by (*R*)-mandelic acid is used in its preparation.
- 2-Amino-5-methoxytetralin is an intermediate in the synthesis of N-0923 (Nagase & Co., Ltd., Japan), a potent dopamine D2 agonist, effective against Parkinson's disease (currently in clinical trial). This intermediate is resolved by diastereomeric salt formation with (*S*)-mandelic acid. The undesired isomer is then racemized and reused in an iterative process.
- In the synthesis of AG-7404 (Pfizer), a key intermediate was resolved by (–)-norephedrine. The final compound was obtained by the combination of diastereomeric and enzymatic resolution.

How to Use ChiroSolv EnantioPrep Kits

The experiment involves 3 phases of work:

1. Screening: Racemate is screened against 384 different combinations of resolving agents and solvents. When the combination of the racemate, resolving agent and solvent is heated together, diastereomeric salt of one enantiomer preferentially crystallizes out after cooling. The best combination of the reagent and solvent that offers the highest yield and enantiomeric enrichment for the target enantiomer is chosen to do further enrichment.
2. Recovery of starting material: After collecting all the material used during the screening process, which includes the racemate, resolving agents and the solvents, this phase will treat it with our "recovery solution" that will separate out the racemate from the resolving agents in form of 2 liquid layers. Using light vacuum pressure, these layers will be separated and the racemate will be recovered from the bottom layer.
3. Enrichment in enantiomeric excess: The goal of this phase is to identify how many re-crystallization steps are required to get the enantiomeric purity needed. The end result should be small quantity of enriched enantiomer (maximum yield: about 30% of the total racemate given).
4. If you prefer to obtain both +ve and –ve enantiomer, add the recovery solution contained in second bottle to the collected filtrate and repeat the steps 2 and 3 above using selected separation condition of that enantiomer.

Note: that there is no guarantee that the enantiomer enrichment will happen; or the target purity will be achieved, since this depends totally on the racemate and the molecules. This product simplifies the work of the chemist and maximizes the probability of good results.

A. Screening

Adding the racemate to the ChiroSolve kits

1. If the racemate is liquid in nature, add 0.03 mmol of racemate into each of the 384 vials of the 4 screen reagent plates using multi-channel pipette; OR
2. If the racemate is solid, dissolve 12 mmol of racemate into the “transfer solvent” (volatile solvent that racemate dissolves in easily). Use minimum amount of solvent needed to dissolve the racemate completely. Dispense equal amounts of this solution into each of the 384 vials of the 4 screen plates using robotic liquid dispenser; or multi-channel pipette; and then add the chiroSolve solvents from the exactly the same position in the solvent plate evaporate out the “transfer solvent” from the kit
3. Add ChiroSolve solvents from the solvent plate into the ChiroSolve kit either by using automatic liquid dispenser, or single/multi-channel pipette

Diastereomeric salt formation

4. Heat the kits to up to 80°C until the mixture in vials become homogeneous. No solid should be visible. Mark the vials with solid present as they may confuse later observations.
5. Cool the kits at room temperature, allowing time for crystals to form, typically overnight.
6. **Note that** depending on the enantiomer property, this may take longer time; and you may need to refrigerate the bottle to maximize the crystal formation
7. Select the vials with crystals for further analysis

Analysis of results

8. For each selected vial with crystals, using the supplied pipette tips with filter, remove the filtrate and collect it in the supplied “filtrate collection bottle”. Wash any crystals collected at the tip with few drops of solvent into the collection reservoir. Analyze the crystals using chiral HPLC.
9. Repeat step 7 for each of the selected vials with crystals and return the vials to the rack
10. Select the best combination of resolving agent and solvent that showed highest enantiomeric enrichment and highest yield as best candidates for the further enrichment steps

B. Sample Recovery

1. Pre-weigh the Enantiomer Collection Bottle without the cover and note the weight.
2. Add 20 µl of recovery solution to each of the 384 vials of the Screen Kit. Heat for 1 minute.
3. Pipette out the content of each of the vials into the Filtrate Collection Bottle.
4. Make sure the final solution in Filtrate Collection bottle is strongly acidic/basic using the pH paper. Add little more “Recovery Solution if needed. Mix well.
5. Transfer the contents of the “Filtrate Recover Bottle” into a Separatory funnel and let the solution stand for few minutes until it separates out into 2 liquid layers.
6. Dispense the bottom layer into “Enantiomer Collection bottle”; discard the top layer containing resolving agents and solvents
7. Attach the filter funnel on top of the Enantiomer Collection bottle and attach the funnel to the vacuum source through its vacuum adapter. Evaporate out the solvents from the racemate
8. Weigh the Enantiomer Collection bottle containing the racemate and determine the recovery yield

C. Enrichment (to get enantiomer with target purity)

1. Based on the amount of racemate recovered, add equal amount (or little less) of the selected resolving agent (chosen during screening) into the Enantiomer collection Bottle. Add 10 ml of the selected solvent (chosen during screening) into the Enantiomer Collection bottle.
2. Heat the Enantiomer Collection bottle in a water bath to up to 80°C, stirring constantly using the magnetic stirrer) until a homogenous mixture is formed. No solid particles should be visible. Add little more solvent if needed.
3. Cool the Enantiomer Collection Bottle at room temperature, allowing time for crystals of enantiomeric salt to form, typically overnight.

Note that depending on the enantiomer property, this may take longer time; and you may need to refrigerate the bottle to maximize the crystal formation

4. Attach the filter funnel on top of the empty Filtrate Collection bottle and attach the funnel to the vacuum source through its vacuum adapter.
5. Pour the mixture with crystals from the Enantiomer Collection Bottle into the filter funnel (with supplied disposable filter and filter out the filtrate into the Filtrate Collection Bottle using low vacuum (5 to 10 psi). Save the filtrate for further recovery of other isomer
6. Using small amount of crystals collected on top of the filter, do HPLC analysis to check how much enrichment has been achieved.
7. In a flask, heat 10 ml of the selected solvent (chosen during screening), close to boiling point
8. Attach the filter funnel on top of the Enantiomer Collection Bottle and pour the solvent in step 7 into the filter funnel. Mix well.
9. Using low vacuum pressure (5 to 10 psi), collect the liquid containing the target enantiomer salt in to the Enantiomer Collection Bottle.
10. Repeat steps 3 to 9 until target (or highest possible) enantiomeric enrichment is achieved.
11. Optionally if you wish to get the other isomer, add the "recovery liquid" from the second recovery bottle to the Filtrate Collection Bottle (that already has the other isomer in collected filtrate) and repeat steps 5 to 9 described in "Sample Recovery" to get as much starting material as possible followed by the Enrichment steps.