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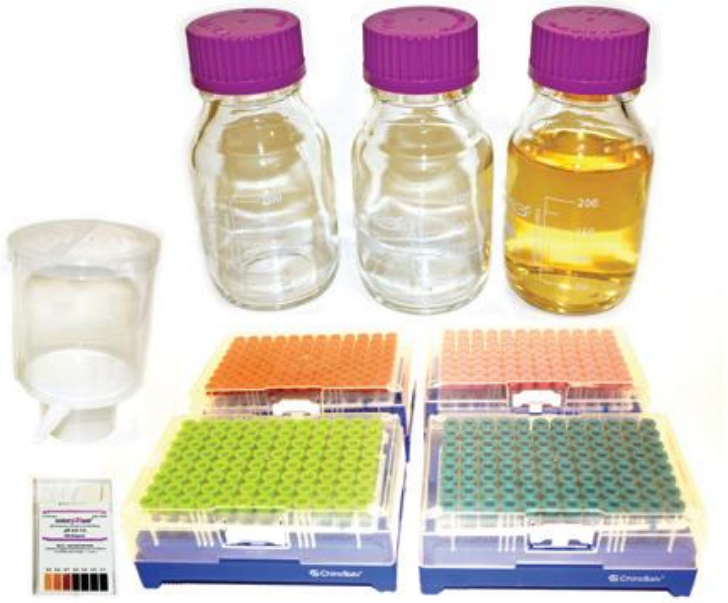
ChiroSolve EnantioPrep Kit-set User's Manual

(to be used with manual kit-set)

DISCLAIMER: this product is a research tool that allows tremendous efficiency in chiral separation process method development. However, since the results are dependent on the properties and nature of the sample racemate being used, ChiroSolve Inc. does not guarantee that the product will always provide the results anticipated by the user in terms of the level of the enantiomeric enrichment; or the recovery of the starting material. The product is for research use only and is not to be used for diagnostic purposes or applications.

Package Content



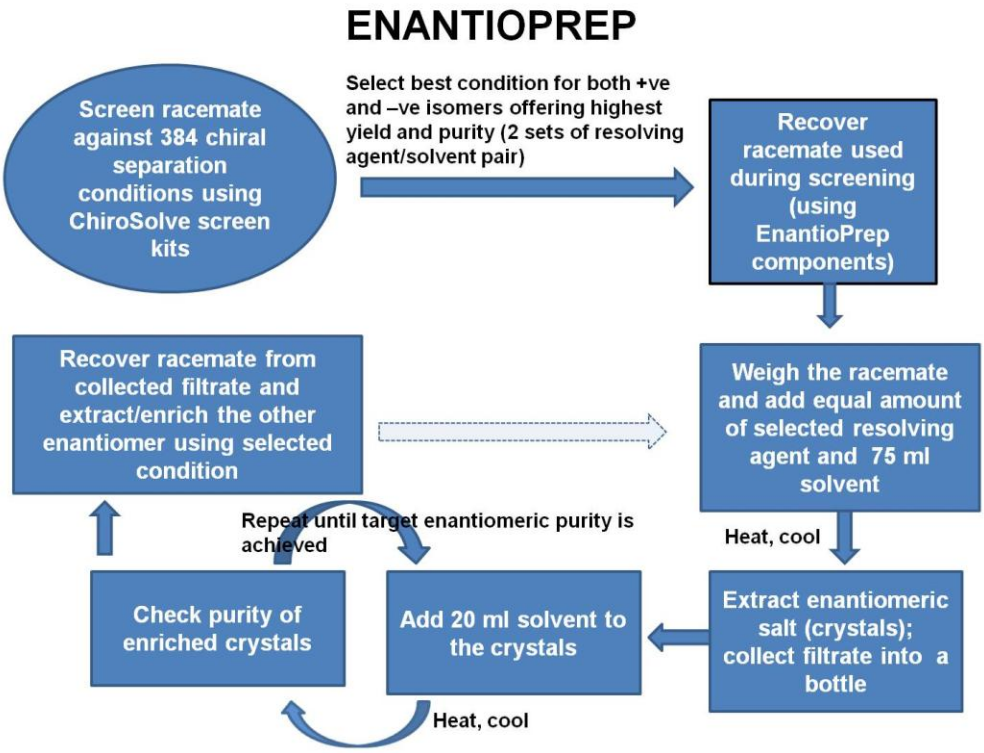
1. ChiroSolve Screen kits for base racemate (A1, A2, A3, A4) ; or for acid racemate (B1, B2, B3, B4)
2. Recovery solution (in two bottles)
3. Filter funnel with PTFE membrane filter and vacuum adapter,
4. disposable filters
5. Enantiomer collection bottle

6. Filtrate collection bottle
7. Pipette tips with filter
8. pH paper

What else will you need?

1. Transfer solvent that the racemate dissolves in (so that you can easily dispense racemate into chiroSolv kits)
2. 12 mmol of resolving agent that is selected after the screening (used during enrichment)
3. 50 ml of solvent that is selected after the screening phase
4. Single/multi-channel pipette; OR robotic liquid dispenser
5. Heating medium and vacuum source
6. Separatory funnel

Procedure



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. **Note that screening identifies ideal separation conditions for both +ve and –ve enantiomer**
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). **Note that you can repeat this phase of work for each of the enantiomer (+ve and –ve) using the ideal separation condition identified during screening.**

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 screening kits 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. **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
6. Select the vials with crystals for further analysis and identify the ideal separation condition for both +ve and –ve enantiomer

Analysis of results

7. For each selected vial with crystals, using a pipette, separate the crystals from the filtrate using pipette. Analyze the crystals as well as the filtrate using chiral HPLC (the 2 readings should validate the results)
8. Repeat step 7 for each of the selected vials with crystals and return the vials to the rack
9. 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. Heat the 4 screening kits to up to 60°C and collect the contents of each of the 384 vials of the 4 screen kits into the separatory funnel
3. Add the recovery solution into separatory funnel until the mixture is strongly acidic (if you are using base kits), or strongly basic (if you are using acid kits). Confirm this using the provided pH paper. Mix well and let it stand until 2 liquid layers form. The top layer contains the resolving agents and solvents; while the bottom layer contains the racemate and solvents
4. Collect the bottom liquid layer into Enantiomer collection bottle and evaporate out the solvent(s). Weight the Enantiomer collection bottle again to determine the amount of racemate recovered (typically 90%)
5. Dispose off the top layer from the separatory funnel

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) to the mixture
2. Heat the Enantiomer collection bottle 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 mixture at room temperature, allowing time for crystals 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. Insert one of the filters into filter funnel (provided) and attach the funnel on top of the empty filtrate collection bottle
5. Pour the mixture with crystals from the Enantiomer collection bottle into the filter funnel and filter out the filtrate. If necessary, attach the funnel to the vacuum source through its vacuum adapter and use very light vacuum (15-20 psi) to collect the filtrate into the filtrate collection bottle. Save the filtrate for further recovery of racemate
6. Using small amount of crystals collected on top of the filter, do HPLC analysis to check how much enrichment has been achieved.
7. Transfer the disposable filter paper into the enantiomer collection bottle
8. In a flask, heat 10 ml of the selected solvent (chosen during screening), close to boiling point
9. Attach the above mentioned filter funnel on top of the Enantiomer collection bottle and pour the solvent into the filter funnel. Mix well.
10. Using low vacuum pressure (5 to 10 psi), collect the liquid containing the target enantiomeric salt into the Enantiomer collection bottle. Mix well and take out the disposable filter paper from the bottle
11. Analyze the enriched purity of the enantiomeric salt formed
12. Repeat steps 3 to 11 until target (or highest possible) enantiomeric enrichment is achieved.
13. Optionally, add the “recovery solution” from the second bottle to the recovery bottle and repeat the steps of (B) and (C) to get the second enantiomer