



Codex™ KRED Panels

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- Functionally diverse KRED variants *demonstrated*:
 - to accept wide range of substrates
 - to produce different stereo-isomers
 - to be chemical process robust (stable to organics, thermo-stable)
 - to be manufactured at commercial scale
- Combinatorial design for sequence-function analysis
- Arrayed on 96-well plates for rapid screening

Advantages to the Process Research Chemist

- Quickly determine feasibility of a biocatalytic route
- Reduce development timelines for biocatalyst optimization – competitive with chemical process optimization
- Available for in-house screening, or as service provided by Codexis
- Deliver low-cost, scalable, environmentally-friendly routes to chiral alcohols

Chiral Alcohols

Chiral alcohols are highly versatile building blocks used in the synthesis of numerous pharmaceutical active ingredients. Traditional methods for their synthesis include use of the available chiral pool, asymmetric catalysis and chiral resolution.

Each of these methods has been used for commercial manufacture of chiral alcohols, but have many drawbacks: use of large volumes of expensive reagents, creation of large amounts of waste and use of extreme process conditions are among the issues that necessitate the development of a more economical and environmentally-friendly method of chiral alcohol production. Biotransformation using either whole-cell or isolated enzyme preparations can overcome many of these issues, but is limited in its commercial application due to typically low yields or inability to perform under standard chemical process conditions.

Codex™ Ketoreductase (KRED) Panels: Biocatalytic Solution to Chiral Alcohol Production

One method to produce chiral alcohols using biotransformation is through the use of Ketoreductases (KREDs). KREDs can selectively reduce ketones to the corresponding chiral alcohol. Codexis now offers a set of >250 unique, proprietary KREDs that are “pre-tuned” to accept a wide range of substrates and to perform under chemical process conditions suitable for pharmaceutical manufacturing. Codex™ KRED Panel biocatalysts are coupled with an optimized cofactor recycling system, and have been shown to perform under a wide range of temperatures (20°C – 45°C), pH (4-11) and solvents (i.e. IPA, nBuOAc, Toluene).

CHIRAL ALCOHOLS PRODUCED USING CODEX™ KRED PANEL TECHNOLOGY

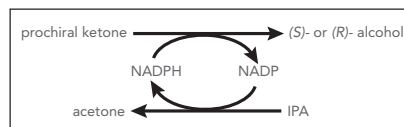
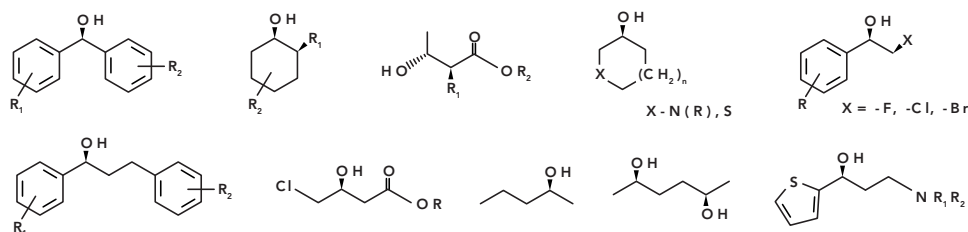


Figure 1 – Lists a number of chiral alcohols produced with high enantiopurity using Codex™ KRED Panel technology.

An Integral Tool for the Process Research Chemist

Screening a Codex™ KRED Panel gives a rapid indication of the feasibility of developing a biocatalytic route for the chiral alcohol of interest. Information on the relationship between enzyme sequence and its performance gained from the Codex™ KRED Panel can guide the design of a “fine-tuned” panel containing biocatalysts further optimized for the particular ketone of interest. This “fine-tuned” panel can be quickly screened to identify high-performing KREDs suitable for process development. Once a project advances, the KRED can be further optimized, if necessary, using Codexis’ proprietary directed evolution platform. Codexis’ KRED biocatalysts have been produced at the level of 100s of

kilograms. The entire process, from initial screen to optimized KRED delivered at kilo scale, takes anywhere from 2-12 months (Figure 2). The result is an economical route suitable for pharmaceutical manufacture.

Codex™ Panels can be integrated at an early stage in process research, when searching for an initial route to generate gram quantities of material for a particular project. Codex™ Panel Biocatalysts can often generate >95% ee for a desired chiral product, and can be quickly scaled to enable initial process research to be conducted. When a project advances and more material is required to be generated at higher efficiencies, information gained

from the Codex™ Panel can be used to generate “fine-tuned” biocatalysts with higher activities and enantioselectivities. When commercial quantities are desired, the biocatalyst can be further optimized to generate, in many cases, ~100% ee at 100-200g/L substrate loading with as low as 1g/L enzyme load. Codex™ Panels can be shipped to the process research chemist to enable in-house screening. Each panel consists of one to three 96-well plates containing cell lysates: simply add substrate, solvent, cofactor (if necessary), allow the reaction to proceed and assay for desired product. Alternatively, Codexis can perform the screen and provide the results.

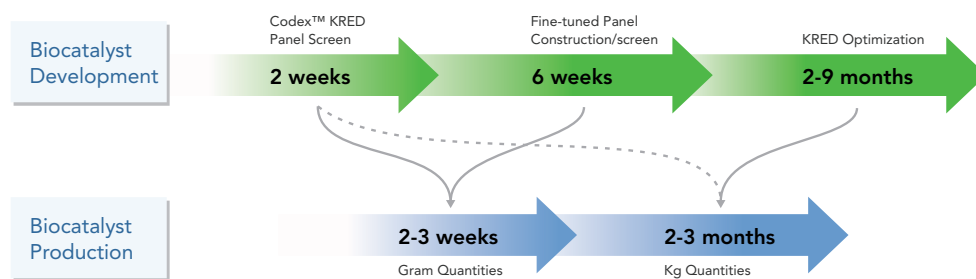


Figure 2 – Time lines for biocatalyst development and production using the Codex™ KRED Panel.

Proven Success

Codex™ KRED Panels have been applied to a number of commercially-relevant projects. The example below (Figure 3) illustrates how the Codex™ KRED Panel was used to rapidly identify a biocatalytic solution for the production of a chiral alcohol used in the manufacture of an intermediate common to a number of carbapenem antibiotics. A key step in the traditional process for this intermediate required the use of an environmentally unfriendly hazardous solvent, high pressure, and an expensive catalyst to produce the desired diastereomer. A Codex™ KRED Panel was screened to identify a biocatalyst with the needed stereospecificity and stereo-selectivity. The best “hit” identified

from the panel showed the desired stereoselectivity for the chiral alcohol, but needed improvements in stereospecificity and volumetric productivity. Sequence-function data obtained from the panel screen pointed to additional mutations that could improve biocatalyst performance. This information was used to optimize the biocatalyst using Codexis’ proprietary directed evolution technology. The final biocatalyst produced the desired chiral alcohol under commercially viable conditions with perfect stereopurity. The entire program, from initial Codex™ KRED Panel screen, to optimized biocatalyst, took 3 months to complete.

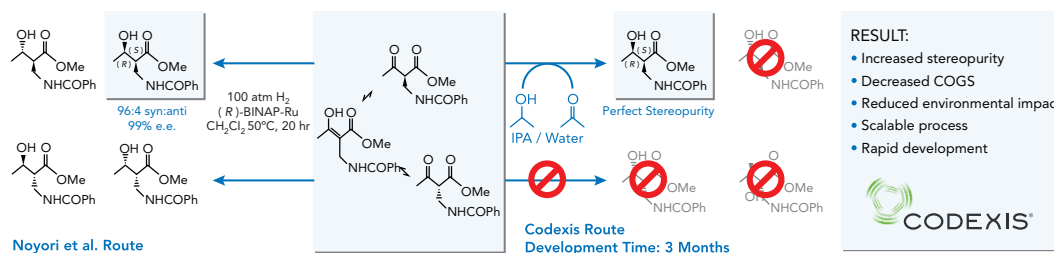


Figure 3 – Codexis process for an intermediate used in the production of carbapenem antibiotics. Using the Codex™ KRED Panels as a starting point, Codexis developed a biocatalytic process for the desired chiral alcohol with perfect stereopurity in only 3 months.