

# Codex™ HDDH Panels

## Codex™ HDDH Panels

- Functionally diverse HDDH 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 epoxides and derivatives

## Chiral Epoxides and Derivatives

Chiral epoxides are highly versatile building blocks used in the synthesis of numerous pharmaceutical active ingredients. Traditional methods for their synthesis include use of asymmetric transition metal catalysts, chiral resolution, and preparation from chiral halohydrins.

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

## Codex™ Halohydrin Dehalogenase (HDDH) Panels: Biocatalytic Solution to Chiral Epoxides and Derivatives

This Codex™ panel plate is based on the most synthetically versatile biocatalyst known: halohydrin dehalogenase (HDDH). HDDHs catalyze the ring closure of a halohydrin to an epoxide and can also catalyze the ring opening of epoxides with anionic nucleophiles. In some cases the epoxide serves as an intermediate and is further elaborated into the final API. A broad range of epoxide ring-opening chemistry can be achieved using the HDDH catalyst, opening the way to derivatives shown in Figure 1. Codexis now offers a set of over 150 unique, proprietary HDDHs that are “pre-tuned” to accept a wide range of substrates and to perform under chemical process conditions suitable for pharmaceutical manufacturing. Codex™ HDDH Panel biocatalysts are cofactor independent and have been shown to perform under a wide range of temperatures (4°C-45°C), pH (5-10) and solvents (i.e. IPA, nBuOAc, Toluene). (For example, refer to Fox, et al., *Nature Biotechnology*, 25, 338-344, 2006).

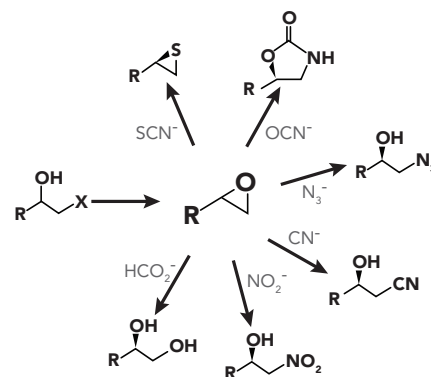


Figure 1 – Scope of reactions catalyzed by the Codex™ HDDH Panel.

## An Integral Tool for the Process Research Chemist

Screening a Codex™ HHDH Panel gives a rapid indication of the feasibility of developing a biocatalytic route for the chiral epoxide or derivative of interest. Information on the relationship between enzyme sequence and its performance gained from the Codex™ HHDH Panel can guide the design of a “fine-tuned” panel containing biocatalysts further optimized for the particular molecule of interest. This “fine-tuned” panel can be quickly screened to identify high-

performing HHDHs suitable for process development. Once a project advances, the HHDH can be further optimized, if necessary, using Codexis’ proprietary evolution platform. Codexis’ HHDH biocatalysts have been produced at the level of 100s of kilograms. The entire process, from initial screen to optimized HHDH delivered at kilo scale, takes anywhere from 2-12 months (Figure 2). The result is an economical route suitable for pharmaceutical manufacture.

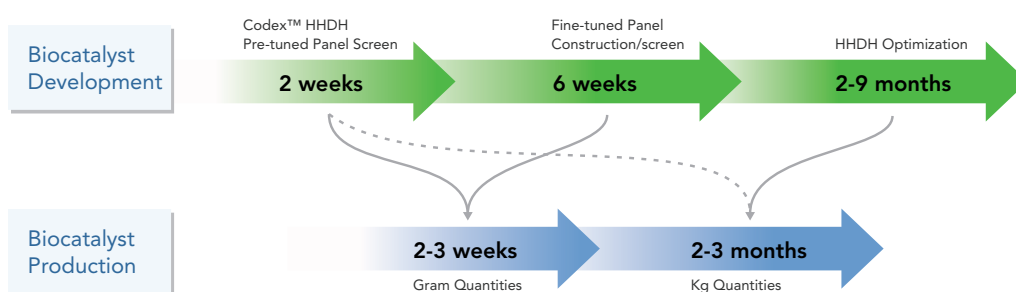


Figure 2 – Time lines for biocatalyst development and production using the Codex™ HHDH Panel.

## Preparing Chiral Alcohols Using Codex™ KRED and/or HHDH Panel Technology

The strength of the Codex™ KRED panel is the fact that a prochiral ketone can be converted into the chiral alcohol in 100% chemical yield and 100% enantiomeric purity. Biocatalytic reduction of a halo-ketone yields the enantiomerically pure halohydrin, which can be sequentially converted using HHDH technology to the epoxide, then cyanoalcohol or azidoalcohol under very mild conditions with high purity (Figure 3a). An additional use of the Codex™ HHDH panel is the conversion of a,α-disubstituted epoxides to derivatized tertiary alcohols – a class of compounds that is not accessible using KREDs (Figure 3b).

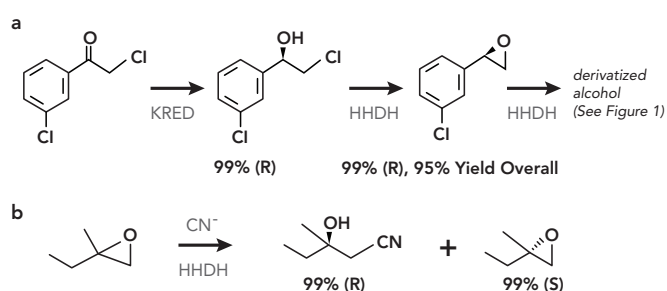


Figure 3 – Preparing chiral alcohols using Codex™ KRED and/or HHDH Panel technology.

## Proven Success

Based on KRED and HHDH technology, Codexis developed a process for ethyl (R)-4-cyano-3-hydroxybutyrate (HN), an intermediate for the statin cholesterol lowering drugs.

The wild-type enzymes all performed below commercialization limits and needed to be improved through directed evolution. The final process combined reactions catalyzed by an optimized KRED, an optimized cofactor-regenerating glucose dehydrogenase (GDH) and an optimized HHDH. The directed evolution has improved the volumetric productivity of the reduction reaction

about 100-fold and that of the cyanation reaction some 4,000-fold. It also involves fewer unit operations than earlier commercial processes for HN, which had required a resolution step with a maximum yield of 50% or syntheses from chiral pool precursors. The HN process is a prime example of an industrial biocatalytic reaction cascade that dramatically decreases the overall cost of the manufacturing process.

Screening the Codex™ KRED and Codex™ HHDH panels will provide an excellent starting point for developing similar processes.

### CODEXIS PROCESS FOR ETHYL (R)-4-CYANO-3-HYDROXYBUTYRATE (HN)

