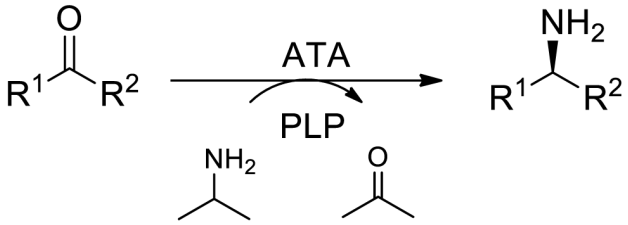
Codex® ATA Screening Kit

Screening Protocol

Reaction of Interest



Codex® ATA Screening Kit General Information

- The Codex® ATA Screening Kit contains 24 -transaminase enzymes (ATAs) that have been selected for their broad substrate range and diverse stereoselectivity for the synthesis of chiral amines from the corresponding ketone and amine donor. This kit is a useful tool to quickly determine the feasibility of using an ATA for an asymmetric transamination.
- The enzymes included in this kit were developed using Codexis' CodeEvolver® protein engineering technology and have been engineered for enhanced selectivity, activity, substrate range, and solvent and temperature stability.
- The 250 mg ATA Screening Kit contains sufficient enzyme to perform ~25 screens using the given protocol. Alternatively, fewer screens can be performed, and the remaining enzyme can be used for confirmation and optimization reactions.
- Recommended storage temperature for the enzyme powders is −20 °C.
- Gram quantities of the ATAs in this kit are available from stock for any follow up work, and kilogram to MT quantities can readily be manufactured upon request for development and commercialization.

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- Codex® ATA enzyme powders, provided in the kit.
- Pyridoxal-5'-phosphate (PLP), provided in the kit.
- Triethanolamine, free base or HCl salt (Sigma catalog #90279 or #T1502, or similar), not provided in the kit.
- Isopropylamine, free base or HCl salt (Sigma #471291), not provided in the kit.
- Solvent, DMSO preferred, not provided in the kit.
- Substrate, approximately 150 mg, not provided in the kit.

Screening Procedure

1. Weigh out approximately 10 mg of each ATA in the kit into separate labeled vials (vials should have at least 1.5 mL total volume). Plastic conical centrifuge tubes of 2 mL volume work well for this as the reaction can be extracted or quenched in the same vial.
2. For each full screen, add the following to a 50 mL vessel:
   a. 20 mL DI water
   b. 1.8 g (2.5 mL) isopropylamine (or 2.9 g isopropylamine-HCl)
   c. 0.5 g triethanolamine (or 0.6 g triethanolamine-HCl)
   d. 8 mg PLP

   Adjust the pH to 7.5 using HCl or NaOH and bring final volume to 30 mL. Final concentrations are 1 M isopropylamine, 0.1 M triethanolamine and 1 mM PLP. Prepare this solution fresh for each screen to avoid decomposition of the cofactor. The solution will be yellow. Add 0.3 mmol of your ketone substrate to 3 mL of DMSO. Mix until dissolved.
3. Add 0.9 mL of the reaction solution from step 2 to the vials containing the ATA enzymes. Mix until enzyme is dissolved. Some cloudiness is acceptable.
4. Begin the reaction by adding 0.1 mL of the substrate-DSMO solution to each vial. It is acceptable if the substrate is not
5. fully soluble under these conditions and typically doesn't hinder the reaction.
6. Mix the reactions at 30 ¬∞C. This is best done by placing the vials horizontally in a shaker, but any method that gives good mixing is acceptable.
7. After ~24 hours analyze your reaction by any preferred method to determine the conversion of ketone to amine. A general protocol follows.

Codex® ATA Screening Kit Work-Up and Analysis

1. Depending on the anticipated method of analysis, follow one of the protocols below:

   a. If using reversed-phase HPLC for analysis, the reaction can be quenched by adding 1 mL of acetonitrile and mixed well to dissolve any insoluble substrate and product.

   b. If using normal phase HPLC or GC for analysis, add 0.1 mL of 5 M NaOH (or similar base to increase reaction pH to >11) and extract into 0.9 mL of ethyl acetate or MTBE.

2. Centrifuge each mixture at ~4000 rpm for 2 min to separate the phases or to sediment any precipitated protein. If a centrifuge is not available, the quenched reaction can be filtered using a syringe filter or, for extractive workup, the phases can be allowed to separate unaided.

3. Transfer the aqueous acetonitrile or the organic phase from each quenched reaction vial to autosampler vials.

4. Analyze for conversion and selectivity by preferred method of analysis. Note: If analyzing by reversed phase HPLC, there will often be a large peak early in the chromatogram corresponding to the PLP cofactor.

Common Screening Questions

(Please see our ATA FAQ sheet for additional information.)

- What if the substrate is very insoluble in water?

  As long as the substrate is soluble in DMSO and can be added equally to each vial, partial insolubility in the reaction mixture is usually not an issue.

- What if low or no activity is found?

  Allow the reaction to run for a longer time, increase the temperature (40 °C) and/or increase the enzyme concentration. Please see the ATA Screening Kit FAQs or contact Codexis to discuss further options of finding a suitable enzyme. You can also consider running a control reaction to verify your reaction solution. Using acetophenone and ATA-025, you can expect to see >30% conversion in 24 hours.

- What if there are too many hits and differences among them cannot be easily determined.

  Stop the reaction at an earlier time or repeat the screen using a higher substrate loading. For the latter, maintain DMSO concentration at <20% v/v.

- What if a hit is identified and the reaction needs to be optimized and scaled up?

  Please see our ATA Screening FAQs for suggestions on optimizing the reaction conditions and scaling up your reaction.

- What if a different amine donor is desired?

  Typical amine donors include alanine, ethylamine, 1- and 2-propylamine, 1- and 2-butylamine and others. The ketone/aldehyde form of these donors also act as good amine acceptors when performing chiral resolution.

Recommended Literature

- Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. Biocatalytic Asymmetric Synthesis of Chiral Amines from Ketones Applied to Sitagliptin Manufacture. Science 2010, 329, 305–309.

- Truppo, M. D.; Rozzell, J. D.; Turner, N. J. Efficient Production of Enantiomerically Pure Chiral Amines at Concentrations of 50 g/L Using Transaminases. Org. Proc. Res. Dev. 2010, 14, 234–237.

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