Codex® ATA Screening Kit

Frequently Asked Questions

WHAT IS THE ATA SCREENING KIT AND WHY USE IT?

- The ATA Screening Kit is a collection of amine transaminases that will provide you with a high chance of finding a hit of meaningful activity and selectivity as the kit enzymes were selected based on the screening results of many projects.
- The enzymes in this kit are always in stock (>50 g) to allow for rapid delivery of follow-on quantities.
- The kit allows you to perform several screens and contains sufficient quantities of each enzyme for immediate scale up. It is typically suitable for determining feasibility and producing >1 g of product.
- Focused screening of just 24 enzymes leads to a shorter total analysis time.

GENERAL INFORMATION

What purity are your enzymes?

- We do not produce our enzymes to a specific purity specification. During production we do separate the enzyme from the majority of the cell debris and other fermentation components. Other compounds such as endogenous proteins and buffer salts may be present and often enhance stability.

What is the shelf life of your enzymes?

- We do not set expiration dates on research enzymes that have not yet been produced at large scale. From experience we have seen that if stored dry at –20 °C, most of the enzymes will remain stable for years.

What is the selectivity of the enzymes?

- The enzymes in the kit are highly selective and split about equally between (*R*) and (*S*) selectivity. We recommend that you screen all the enzymes rather than depend upon historical notation as the substrates can fit into the active site in unusual conformations. Note that the presence of non-carbon atoms in the substrate (such as halogens or sulfur) may give the opposite selectivity due to Cahn-Ingold-Prelog isomer naming conventions.

What are the Typical Parameters under which the enzymes work?

- The stability and activity of the ATAs are affected by several interdependent factors and can vary with different substrates and products. The table below gives typical performance parameters that might aid further optimization after screening.

Enzyme	Maximum [DMSO] (v/v)	Maximum Temperature	Optimal pH Range	Typical Selectivity
ATA-007	10%	30 °C	7–8.5	R
ATA-013	10%	35 °C	7–8.5	R
ATA-025	50%	50 °C	7–9	R
ATA-113	10%	30 °C	7–8.5	S
ATA-117*	10%	30 °C	7–8.5	R
ATA-200	10%	35 °C	7–8.5	S
ATA-217	20%	45 °C	7–9	S
ATA-234	25%	55 °C	7–9	S
ATA-237	20%	45 °C	7–9	S
ATA-238	25%	55 °C	7–9	S
ATA-251	20%	45 °C	7–9	S
ATA-254	20%	45 °C	7–9	S
ATA-256	20%	50 °C	7–9	S
ATA-260	20%	50 °C	7–9	S
ATA-301	40%	45 °C	7–10	R
ATA-303	40%	45 °C	7–10	R
ATA-412	50%	45 °C	7–10	R
ATA-415	20%	45 °C	7–10	R
ATA-P1-B04	10%	35 °C	7–8.5	S
ATA-P1-F03	10%	35 °C	7–8.5	S
ATA-P1-G05	10%	35 °C	7–8.5	S
ATA-P2-A01*	10%	30 °C	7–8.5	R
ATA-P2-A07*	10%	30 °C	7–8.5	R
ATA-P2-B01*	10%	30 °C	7–8.5	R
ATA-012^	10%	35 °C	7–8.5	R
ATA-024^	50%	50 °C	7–9	R
ATA-P1-A01^	10%	35 °C	7–8.5	S
ATA-P1-G06^	10%	35 °C	7–8.5	S

*Not sold in Japan, replacement enzymes are denoted with ^.

PLANNING YOUR SCREENING EXPERIMENT

What should I use as a control substrate?

- Acetophenone is a general substrate for most of the enzymes in this kit. If you would like to test your reaction system, expect ATA-025 to give >30% conversion with acetophenone when used as described in the screening protocol.

What are typical reaction conditions for the evolved enzymes?

- 1–200 g/L substrate, 100 mM triethanolamine hydrochloride, 1 mM PLP, 1 M isopropylamine (IPM-HCl), pH 7.5.

What buffers can be used with the enzymes?

- We suggest screening in triethanolamine hydrochloride buffer at pH 7.5. Once hit(s) are identified, the buffer type and pH can be optimized. Selectivity is typically not pH dependent, however activity may be. Borate buffer can be used to evaluate performance at high pH.

What solvents can be used with the enzymes?

- Over 50% of the kit enzymes are stable towards up to 20% v/v DMSO as co-solvent, see the **Typical Parameters** table for specific values. All the enzymes generally tolerate ~10% v/v ethanol, isopropanol, methanol, acetonitrile or THF.

What is the tolerance to pH?

- All enzymes operate between a pH of 7 and 8.5. A small subset is tolerant to pH 10. We do not recommend decreasing the pH below 7.0. See the **Typical Parameters** table for more details.

What is the tolerance to temperature?

- The variants have differing stability but range between 30 °C and 55 °C. See the **Typical Parameters** table for more details.

COMMON SCREENING QUESTIONS

I prepared my buffer/cofactor solution last week. Can I still use it?

We recommend that you make up your solutions fresh on the day of the experiment. The substrate and cofactor may degrade over time, decreasing the overall performance of the reaction. If the solution must be stored and reused, it should be kept cold and protected from light and, if possible, the performance checked against fresh solutions.

What can I do if substrate solubility in aqueous media is a challenge and the reaction mixture is cloudy?

- For many of the enzymes, 20% v/v DMSO can be used and the temperature can also be increased to 40–45 °C to increase solubility. See the **Typical Parameters** table for typical conditions tolerated for each enzyme.

- Increasing the pH may also help, but this is substrate dependent. Borate buffer can be used instead of triethanolamine at high pH. We do not recommend decreasing pH below 7.0.
- Cloudiness indicating that the substrate is not fully soluble in the reaction mixture is acceptable and typically does not hinder the reaction. It is best to add the substrate neat or from a DMSO (or similar solvent) solution to ensure it is added evenly to all reactions.

Is there an order in which reagents should be added?

- It is typically best to add the enzyme after all the other reagents and solvents have been added and the pH had been adjusted. If possible, first dissolve the enzyme in buffer before adding to the reaction mixture. Occasionally, changing the order of reagent addition can have a drastic effect on reaction performance (either beneficially or deleteriously).

OPTIMIZATION

What is the best way to optimize the reaction conditions (temperature, pH, co-solvent, isopropylamine concentration, substrate/enzyme ratio)?

- It is important to understand the inherent nature of the substrate and product since those are properties that the enzyme cannot affect (*e.g.* solubility, pH and temperature stability). In addition, you will want to avoid conditions that promote side reactions, create downstream issues or are otherwise incompatible with your substrate or product.
- Evaluate the performance of the reaction at different pH levels (pH 7.5, 9 and 10 are recommended using triethanolamine at pH 7.5 and sodium borate at pH 9 and 10).
- Once an optimal pH is found, investigate the effect of temperature on the reaction performance (recommended temperature range is 30–55 °C).
- Evaluate the reaction at higher substrate loadings. Suggested conditions are 5 g/L ATA and substrate loadings ranging from 5 to 100 g/L.
- Ensure that the isopropylamine (IPM) concentration is high enough that it is never limiting (especially important at high substrate loadings). Typically 1 M should be sufficient. Excess IPM is often required to drive equilibrium to high conversion (see below).
- If high conversion is obtained at high substrate loadings (50–100 g/L), then investigate reducing the ATA concentration (recommended range is 1–5 g/L).
- If the substrate is not soluble in the reaction mixture, substrate mass transfer limitation may occur. In this case, addition of a solubilizing co-solvent should be investigated. See the **Typical Parameters** table for solvent concentration tolerance (in addition to DMSO, other solvents such as short chain alcohols, DMF, THF and acetonitrile can also be investigated).
- See the **Typical Parameters** table for general tolerances of each ATA in the kit. It may be possible to exceed these tolerances depending on the particular reaction being performed.

What if the reaction appears to stall after a certain time or conversion?

- If the reaction seems to stall at a particular conversion, it is important to understand that a transamination is an equilibrium reaction, and often requires a driving force to achieve high conversion. This can be accomplished by using a high isopropylamine concentration (relative to the ketone substrate) and/or by removal of the acetone by-product. The latter can be achieved by applying a partial vacuum or by sweeping nitrogen across the surface of or through the reaction. This will result in some concurrent loss of isopropylamine and pH drop. The reaction pH should be monitored and if the pH decreases, it can be increased by drop wise addition of a 50% aqueous solution of IPM until the desired pH is obtained.
- The reaction may also stall due to enzyme inactivation under the given reaction conditions. This can be elucidated by adding fresh enzyme after the reaction has stalled and observing an increase in conversion.

SCALE-UP

What should I do once I find hits under screening conditions and want to scale up the reaction?

- Scaling up an ATA reaction is very similar to the procedure used for screening the ATAs. You will want to do at least some optimization first (see previous section) to determine optimal substrate and enzyme loading, co-solvent concentration, pH, temperature, and reaction time. Some general guidelines are listed below.

What conditions should I use to scale up an ATA reaction?

- Typical reaction conditions, which may change after optimization, are:
  - Ketone substrate: 10–100 g/L
  - ATA: 1–10 g/L
  - Isopropylamine: 1–2 M
  - PLP: 1 mM
  - Co-solvent: 0–20% DMSO, other solvents can also be evaluated
  - Buffer: 100 mM, pH 7–10 (triethanolamine, potassium phosphate, or sodium borate)

What is a typical reaction procedure for scaling up an ATA reaction?

1. Combine all reaction components except for the enzyme (retain some buffer for step **3**) and mix well until dissolved. If substrate will not fully dissolve, mix until well dispersed and any large masses of substrate have been broken up. If using a co-solvent such as DMSO it is often helpful to first dissolve the substrate in the co-solvent before adding it to the reaction buffer. If after mixing the substrate is not fully soluble, that is acceptable and typically does not hinder the reaction.

2. Check the pH of the substrate solution/mixture and adjust to the desired pH, if necessary.

3. Dissolve the ATA in the retained buffer. Enough buffer should be retained from step **1** such that the enzyme concentration in this step is <50 g/L.

4. Add the enzyme solution in step **3** to the substrate solution/mixture in step **1** and mix well. Check pH and adjust if necessary; avoid using concentrated acid or base for pH adjustment when enzyme is present. Typically 1 M acid or base (HCl or K/NaOH) concentration is sufficient. The pH adjustment should be done while the reaction is stirring.

5. Stir the reaction at the desired temperature. Ensure that the mixing is adequate, especially if the substrate is not fully soluble. However, vigorous mixing such that the reaction produces foam should be avoided.

6. The reaction time course can be monitored by taking in-process check (IPC) samples periodically (e.g. 2, 4, 6, 16, and 24 hrs.). Samples can be quenched with 1–2 volumes of acetonitrile or methanol or extracted with 1–2 volumes of ethyl acetate or MTBE prior to analysis (if extracting, IPC samples should be basified to pH >11 with K/NaOH prior to extraction). If the substrate has limited solubility in the reaction mixture, it can sometimes be difficult to get representative IPC samples and it is suggested multiple IPC samples be taken at each time point and averaged.

7. If the reaction has not reached high conversion (>80–90%) after 24 hrs., or if there is no difference in conversion between 16 and 24 hrs., additional ATA, isopropylamine, and/or PLP can be added. Generally adding 0.5–1 equivalent of what was initially used is usually sufficient to determine if this component is limiting the reaction. Samples can be checked after 4–6 hrs. to determine if this addition improved the reaction conversion.

MISCELLANEOUS

What do you suggest if I am trying to resolve a racemic amine and want to run the reaction in the deamination direction?

- For the deamination reaction, we suggest starting with the following reaction conditions.
  - 5 g/L amine substrate
  - 1 mM PLP
  - ~5 equivalents of acetone (<1% v/v)
  - 15 g/L enzyme loading

What do you suggest if I am trying to run a dynamic kinetic resolution?

- The basic protocol remains the same, but conditions that increase the racemization rate of the non-amine stereocenter may improve the selectivity and rate. These conditions include increasing the reaction pH, the substrate solubility (by increasing co-solvent concentration) and increasing reaction temperature. It is important that the conditions used are not detrimental to the substrate or product.

If I need help, is technical support available?

- Yes! Feel free to contact us at sales@codexis.com and we will be happy to assist you.