JM

Product guide: Transaminase

JM Johnson Matthey

The enzymes in this kit have been selected for their activity on a wide selection of substrates and in a wide range of pH conditions to meet your specific synthetic needs.

Aromatic and aliphatic primary amines can be obtained using our transaminases.

The results of the TAs screening will depend on the inherent enzyme substrate scope and substrate properties. If the amine moiety in the product molecule is stabilised by hydrogen bonds with other neighbouring functionalities, the reaction will reach higher conversions because the concurrent deamination reaction is less favoured.

Also provided in this kit are: 1 LDH, 1 AlaDH, 1 GDH (see separate leaflet), 350 mg NAD^+ and 100 mg PLP.

Kit description - chiral amines kit (EZK004)

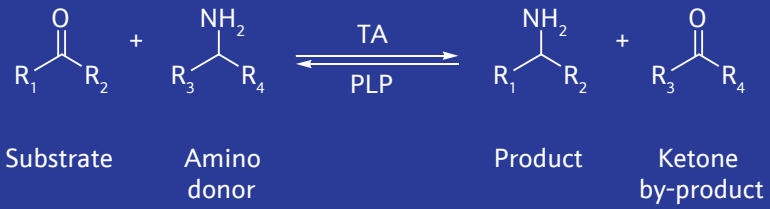
This kit contains 18 transaminase (TA) enzymes for the formal reductive amination of ketones to the corresponding (*R*)- or (*S*)-amine. The kit contains 10 (*R*)-transaminases and 8 (*S*)-transaminases, using methylbenzylamine as the product reference. The TAs in this kit include wild-type and engineered enzymes.

TAs require a PLP cofactor for catalysing the reaction. PLP binds in the active site of the transaminase and is instrumental in catalysing the transfer of the amine from an amino donor to the substrate, hence forming the desired chiral amine.

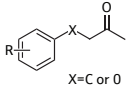
For the transamination to work, an amino donor has to be added to the reaction: common amino donors are isopropylamine and alanine, although other small amines can be used (i.e. 2-butylamine, methylbenzylamine, cadaverine).

The TA-catalysed reaction is an equilibrium. For the desired amination to occur, it may be necessary to apply methods that shift the equilibrium toward the desired product, such as using an excess of amino donor or an additional enzyme that reacts with the ketone by-product (see useful tips for more details).

Since the reaction is an equilibrium, TAs are also successfully used to deracemise amines. In this case, pyruvate is added as the co-substrate, forming alanine as a by-product.



Enzyme overview

Enzyme	Cofactor	Optimum pH	Amino donor		 X=C or 0	
STA-1	PLP	8.0 – 9.0	IPA and L-Alanine	++	++	++
STA-2	PLP	6.5 – 7.5	IPA and L-Alanine	++	++	++
STA-13	PLP	6.0 – 8.5	IPA and L-Alanine	+	++	-
STA-14	PLP	6.5 – 10.0	IPA and L-Alanine	+	++	++
STA-113	PLP	8.0 – 8.5	IPA and L-Alanine	+	++	-
STA-118	PLP	8.5 – 9.0	IPA and L-Alanine	++	++	+
STA-120	PLP	8.0 – 8.5	IPA and L-Alanine	-	+	+
STA-121	PLP	8.0 – 8.5	IPA and L-Alanine	-	++	+
RTA-25	PLP	6.5 – 10.0	IPA and D-Alanine	+	-	-
RTA-40	PLP	7.0 – 8.0	D-Alanine	+	++	++
RTA-45	PLP	6.5 – 7.5	IPA and D-Alanine	++	+	+
RTA-57	PLP	6.0 – 7.0	IPA and D-Alanine	+	++	+
RTA-58	PLP	7.5 – 8.0	D-Alanine	++	+	+
RTA-102	PLP	7.0 – 7.5	IPA and D-Alanine	+	++	+
RTA-103	PLP	7.5 – 8.0	IPA and D-Alanine	-	++	+
RTA-104	PLP	7.5 – 8.5	IPA and D-Alanine	+	++	+
RTA-105	PLP	7.0 – 8.0	IPA and D-Alanine	+	++	++
RTA-194	PLP	7.0 – 8.0	IPA and D-Alanine	+	++	++

Key

++ Good conversion + Moderate conversion - Poor conversion

Reaction setup and work-up

1. Weigh 5 mg of each TA in a microcentrifuge tube or reaction vial.
2. In a separate container, prepare the reaction mix:
   - For isopropylamine (IPA) as amino donor: dissolve 2.5 mg PLP (1 mM) in 9 mL 1 M IPA buffered solution at pH 7.5.
   - For alanine as amino donor: dissolve 2.5 mg PLP (1 mM), 78.4 mg racemic alanine (88 mM), 78.4 mg D-glucose (44mM), 6.8 mg NAD^+ (1 mM), 10 mg GDH-101 (1 mg/mL), and for shifting the reaction equilibrium, 20 mg of LDH (2 g/L) in 9 mL buffer 100 mM, ph 7.5.
3. Add 450 µL reaction mix to the enzyme powder.
4. Add 50 µL of substrate of choice from a DMSO or toluene stock, containing 200 mM ketone (final substrate concentration in the reaction 20 mM).
5. Incubate the reaction at 30-35 °C for 18 hours in a thermostated shaker or with magnetic stirring.
6. Work-up the reactions for GC or HPLC analysis: basify with NaOH to pH >10 and extract with organic solvents (i.e. EtOAc extraction with 600 µL twice) for GC or dissolve the reaction with 1 mL acetonitrile for HPLC.

Useful tips

- Most TAs are active at pH 7.5 therefore this is the recommended pH for initial screening.
- The preferred buffer for TAs is HEPES-HCl: to prepare 500 mL of 100 mM HEPES buffer, weigh 11.9 g HEPES, dissolve in milli-Q water and adjust to the desired pH with HCl.
- For simplicity, initial screening can also be carried out in potassium phosphate buffer pH 7.5: to prepare 500 mL of 100 mM potassium phosphate buffer mix 7.26 g K_2HPO_4 and 1.13 g KH_2PO_4 in milli-Q or distilled water.
- For IPA as amino donor prepare 500 mL of 1 M IPA in buffered solution, add 25 mL IPA to 400 mL buffer, adjust to desired pH with HCl and add buffer until 500 mL.
- As an alternative to LDH, AlaDH can be used for equilibrium shift. In this case, add to the reaction mix 20 mg AlaDH and 11.8 mg NH_4Cl (22 mM).
- The TAs in the JM kit have different preferences for IPA or alanine as amino donor. Therefore for an initial screening it is recommended to try both systems to maximise the chances of finding an active enzyme.

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