

# Twist-bitBiome Transaminase Kit

## Enzyme Activity Protocol

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The Twist-bitBiome Transaminase Kit can be used to screen a panel of transaminase enzymes for effectiveness and efficiency in catalyzing reactions for amine synthesis. This kit provides 48 validated, unique, and highly active transaminase candidates. The chosen enzymes represent a diverse set of substrate specificities including bulky substrates and function in a wide array of temperatures and pH environments. Each enzyme in this kit is supplied at 5 mg allowing them to be used in up to 10 reactions with the provided protocol.

These candidates were selected from bitBiome's unique metagenomic database bit-GEM using a proprietary enzyme discovery platform supported by Twist Bioscience's high-throughput DNA synthesis capability. Through this discovery pipeline, we have identified many enzymes that maintain activity in a variety of extreme conditions and are useful for various applications including pharmaceutical manufacturing. High-activity enzymes found with this kit can be used as is or taken through an enzyme refinement process leveraging the aforementioned discovery platform for scaling up production or further developing and optimizing the enzymes of choice.

This manual details all the steps, reagents, and equipment required to conduct a transaminase activity experiment using the Twist-bitBiome Transaminase Kit. The protocol has been optimized for use with the reagents specified and should only be performed with them or equivalent molecular biology grade reagents.

## PROTOCOL COMPONENTS

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Please read the product packaging and storage recommendations carefully for each kit and store components as recommended immediately upon arrival.

CATALOG #	NAME	DESCRIPTION	STORAGE
108622	Twist-bitBiome Transaminase Kit	Plate with 48 validated and highly active transaminases. Also included are plates of reagents and amine acceptors.	-20°C

*For Research Use Only. Not intended for use in diagnostic procedures.*

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## INTENDED USE

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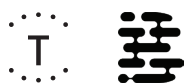
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## TABLE OF CONTENTS

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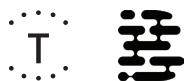
Twist-bitBiome Transaminase Kit Enzyme Activity Protocol	1
Materials Supplied by User	4
General Notes and Precautions	5
Guidelines	6
Planning a Screening Experiment	11
Control Transaminase Activity Protocol	12
Transaminase Activity Protocol	14
Appendix	16
FAQ for Quality Control of Enzyme Kit Manufacturing	17



## MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to perform a transaminase activity experiment using the Twist-bitBiome Transaminase Kit

PRODUCT	SUGGESTED SUPPLIER
<b>REAGENTS AND CONSUMABLES</b>	
Molecular biology grade water	Cytiva, cat. SH30538.03
Potassium phosphate, 0.5 M buffer soln., pH 7.4, CAS 7778-77-0 or Other buffers of interest	Thermo Fisher Scientific, cat. J61413.AK
Sodium Pyruvate, CAS 113-24-6 or Other amine acceptors of interest	Thermo Fisher Scientific, cat. BP356-100
2-(4-Nitrophenyl)ethylamine hydrochloride, 98+%, CAS 29968-78-3 or Other amine donors of interest	Thermo Fisher Scientific, cat. H64894.06
Pyridoxal 5-Phosphate Monohydrate, >98.0%, CAS 41468-25-1	TCI, cat. C0377
Dimethyl sulfoxide (DMSO), CAS 67-68-5 or Other substrate solvents of interest	Thermo Fisher Scientific, cat. BP231-100
<b>EQUIPMENT</b>	
Pipettes and tips	—
Clear flat-bottom 96-well microtiter plate	Greiner, cat. 655801
Plate reader supporting A490 absorbance (optional)	Molecular Devices
Microplate shaker supporting 1000 rpm agitation	Eppendorf



## GENERAL NOTES AND PRECAUTIONS

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Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol and follow the provided instructions. Twist and bitBiome cannot guarantee the performance of the Twist-bitBiome Transaminase Kit if modifications are made to the protocol.

The enzymes in this kit are continuously improved and thus are not produced to a specific activity or purity standard. However, the activity of each enzyme lot is tested to ensure there is a significant color change, as measured by A490 readings, after a 16-hour reaction at 30°C. Please see the FAQ for further details. Additionally, while efforts are made to separate the enzymes from cell debris and fermentation components, some endogenous proteins and buffer salts may be present. These additional components often enhance stability.

The kit can be stored unopened at -20°C for up to 2 years.

Enzyme powders should be stored dry as moisture can reduce activity.

We recommend preparing solutions fresh on the day of the experiment. Over time, both the substrate and cofactor may degrade, potentially reducing the overall performance of the reaction.

This kit comes with enough control substrates and buffers for 10 reactions. If more reactions are needed, the user must supply the additional materials.

# GUIDELINES

## SUBSTRATE SPECIFICITY

The enzymes in the kit exhibit high selectivity, splitting between (R) and (S) selectivity. We advise screening all enzymes rather than relying solely on the reference data in Tables 1 and 2, as substrates may adopt unusual conformations that affect selectivity. It is important to note that the presence of non-carbon atoms in the substrate (e.g., halogens or sulfur) may lead to opposite selectivity due to Cahn-Ingold-Prelog isomer naming conventions. Table 3 provides the structure and CAS number identification for each substrate listed in Tables 1 and 2.

**Table 1. Substrate Specificity (Columns 3 - 6)**



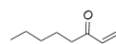
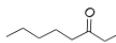
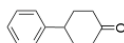
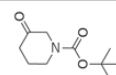



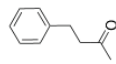
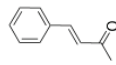
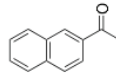



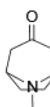
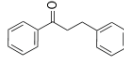
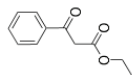
WELL	ENZYME	SUBSTRATE SPECIFICITY																	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
B3	TA0093	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-
C3	TA0452	+	+	+	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-
D3	TA0227	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
E3	TA1313	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-
F3	TA1441	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	+	-
G3	TA1452	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-
B4	TA0491	+	+	+	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-
C4	TA0453	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
D4	TA0338	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
E4	TA0772	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-
F4	TA1451	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
G4	TA0821	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
B5	TA0697	+	+	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-
C5	TA0535	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	-	-
D5	TA0488	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
E5	TA1391	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-
F5	TA0817	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G5	TA1318	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-
B6	TA0646	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
C6	TA0196	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
D6	TA0643	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-
E6	TA1062	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
F6	TA1443	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G6	TA1439	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-

Table 2. Substrate Specificity (Columns 7 - 10)

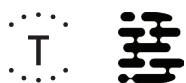
WELL	ENZYME	SUBSTRATE SPECIFICITY																	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
B7	TA0740	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
C7	TA0219	+	+	+	-	+	+	+	+	+	-	+	+	-	-	-	-	-	-
D7	TA0677	+	+	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-
E7	TA1450	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
F7	TA1317	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-
G7	TA1444	+	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-
B8	TA0289	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-
C8	TA0252	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-
D8	TA0729	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
E8	TA0781	+	+	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-
F8	TA1375	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G8	TA1420	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
B9	TA0567	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
C9	TA0537	+	+	+	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-
D9	TA1312	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-
E9	TA1060	+	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-
F9	TA1402	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-
G9	TA1446	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
B10	TA0665	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
C10	TA0724	+	+	+	-	+	+	+	-	+	+	+	+	-	-	-	-	-	-
D10	TA1447	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E10	TA1316	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-
F10	TA1442	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-
G10	TA1457	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-

\*This substrate should be dissolved in water

**Table 3. Substrate Identities**

	SUBSTRATE NAME	CAS NUMBER	STRUCTURE
<b>A</b>	Sodium Pyruvate	113-24-6	
<b>B</b>	Butyraldehyde	123-72-8	
<b>C</b>	1-Octen-3-one	3391-86-4	
<b>D</b>	3-Octanone	106-68-3	
<b>E</b>	4-Phenylcyclohexanone	4894-75-1	
<b>F</b>	1-(tert-Butoxycarbonyl)-3-piperidone	98977-36-7	
<b>G</b>	2-Acetylpyrazine	22047-25-2	
<b>H</b>	Acetophenone	98-86-2	
<b>I</b>	2-Acetylpyridine	1122-62-9	
<b>J</b>	4-Phenyl-2-butanone	2550-26-7	
<b>K</b>	trans-Benzalacetone	1896-62-4	
<b>L</b>	2-Acetonaphthone	93-08-3	
<b>M</b>	1-Indanone	83-33-0	
<b>N</b>	(-)-Camphor	464-48-2	
<b>O</b>	(+)-Camphor	464-49-3	
<b>P</b>	Tropinone	532-24-1	
<b>Q</b>	1,3-Diphenyl-1-Propanone	1083-30-3	
<b>R</b>	Ethyl Benzoylacetate	94-02-0	





## ENZYME REACTION CONDITIONS

The stability and activity of transaminases can vary due to several interdependent factors, including different substrates and products. Table 4 below provides enzyme performance parameters that may assist in further optimization after screening.

**Table 4. Enzyme Typical Parameters**

WELL	ENZYME	OPTIMAL TEMPERATURE (°C)	OPTIMAL pH RANGE	TYPICAL SELECTIVITY
B3	TA0093	40	7-8.92	S
C3	TA0452	20	7-7.63	S
D3	TA0227	40	7-9.27	S
E3	TA1313	60	7-9.27	S
F3	TA1441	30	7-7.64	R
G3	TA1452	40	7-9.27	R
B4	TA0491	30	7-7.63	S
C4	TA0453	30	7-8.26	S
D4	TA0338	30	7-7.63	S
E4	TA0772	30	7-8.92	S
F4	TA1451	20	7-7.63	R
G4	TA0821	30	7-9.27	S
B5	TA0697	30	7-8.26	S
C5	TA0535	30	7-7.63	S
D5	TA0488	30	7-8.26	S
E5	TA1391	20	7-7.63	S
F5	TA0817	50	7-8.92	S
G5	TA1318	40	7-7.63	S
B6	TA0646	30	7-9.27	S
C6	TA0196	30	7-10.1	S
D6	TA0643	30	7-8.26	S
E6	TA1062	30	7-9.27	S
F6	TA1443	30	7-8.92	R
G6	TA1439	50	7-10.1	R

(continued on next page)



WELL	ENZYME	OPTIMAL TEMPERATURE (°C)	OPTIMAL pH RANGE	TYPICAL SELECTIVITY
B7	TA0740	30	7-8.92	S
C7	TA0219	30	7-8.92	S
D7	TA0677	30	7-8.26	S
E7	TA1450	20	7-7.63	<b>R</b>
F7	TA1317	30	7-7.63	S
G7	TA1444	30	7-8.26	<b>R</b>
B8	TA0289	40	7-8.26	S
C8	TA0252	40	7-9.27	S
D8	TA0729	20	7-9.27	S
E8	TA0781	30	7-8.26	S
F8	TA1375	30	7-10.1	S
G8	TA1420	20	7-7.63	<b>R</b>
B9	TA0567	20	7-7.63	S
C9	TA0537	30	7-9.27	S
D9	TA1312	50	7-10.1	S
E9	TA1060	20	7-8.26	S
F9	TA1402	30	7-8.26	<b>R</b>
G9	TA1446	20	7-10.1	<b>R</b>
B10	TA0665	30	7-8.26	S
C10	TA0724	40	7-9.27	S
D10	TA1447	30	7-7.63	<b>R</b>
E10	TA1316	40	7-8.26	S
F10	TA1442	20	7-10.1	<b>R</b>
G10	TA1457	30	7-8.26	<b>R</b>

### Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			TA0093	TA0491	TA0697	TA0646	TA0740	TA0289	TA0567	TA0665		
C			TA0452	TA0453	TA0535	TA0196	TA0219	TA0252	TA0537	TA0724		
D			TA0227	TA0338	TA0488	TA0643	TA0677	TA0729	TA1312	TA1447		
E			TA1313	TA0772	TA1391	TA1062	TA1450	TA0781	TA1060	TA1316		
F			TA1441	TA1451	TA0817	TA1443	TA1317	TA1375	TA1402	TA1442		
G			TA1452	TA0821	TA1318	TA1439	TA1444	TA1420	TA1446	TA1457		
H												



## PLANNING A SCREENING EXPERIMENT

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Pyruvic acid serves as a general amine acceptor for most enzymes in this kit, so it is an ideal control substrate for testing your reaction system.

If a different amine donor than the one listed in the protocol is desired, potential alternate amine donors include alanine, ethylamine, 1- and 2-propylamine, 1- and 2-butylamine, methylbenzylamine, and others. The ketone/aldehyde forms of these donors also act as good amine acceptors during chiral resolution. Please see the section Optimizing Reaction Conditions when using alternate donors and acceptors.

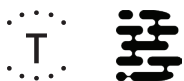
For all enzymes in this kit, typical reaction conditions include 25 mM amine acceptor, 25 mM amine donor, 100 mM potassium phosphate buffer (pH 7.4), and 1 mM pyridoxal 5-phosphate (PLP) as a cofactor.

We recommend screening in potassium phosphate buffer at pH 7.4 initially. Once hit(s) are identified, buffer type and pH can be optimized. Selectivity is typically not pH dependent, though activity may vary. Borate buffer is suitable for evaluating performance at high pH.

Most of the enzymes in the kit remain stable with up to 10% v/v DMSO as a co-solvent.

Enzymes in the kit operate effectively within a pH range of 7 to 8.5, with a small subset tolerant at pH 9 to 10. It's not recommended to decrease the pH below 7.

The enzymes exhibit differing stability but generally operate between 20°C and 60°C. Refer to the Enzyme Typical Parameters table for more details.



## STEPS

## CONTROL TRANSAMINASE ACTIVITY PROTOCOL

The following section outlines the steps necessary to carry out a control transaminase activity experiment.

### Consumables and Reagents

- Enzyme Lysate Plate
- Smart Reagent Plate
- Smart Amine Acceptor (Pyruvate)
- Molecular biology grade water

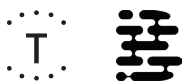
### Before You Begin

- Pulse spin the Enzyme Lysate Plate and the Smart Reagent Plate at 1500 xg for 1 minute to collect the reagents. Let both plates equilibrate to room temperature for 30 minutes.

## WORKFLOW

- C.1** Prepare enzyme solutions by adding 100  $\mu$ l molecular biology grade water to each well in the Enzyme Lysate Plate. Seal and shake the plate at room temperature for 5 minutes with 1000 rpm agitation using a microplate shaker.
- C.2** Centrifuge the Enzyme Lysate Plate at 1500 xg for 1 minute to collect the enzyme solutions and then place the enzyme plate on ice.
- C.3** Rehydrate each smart reagent in the Smart Reagent Plate with 177.5  $\mu$ l of molecular biology grade water. Vortex the reagent plate until the powders are completely dissolved.
- C.4** Centrifuge the Smart Reagent Plate at 1500 xg for 1 minute to collect the smart reagent solutions and then place the reagent plate on ice.
- C.5** Rehydrate the Smart Amine Acceptor (Pyruvate) with 660  $\mu$ l of molecular biology grade water and vortex until the powder is completely dissolved. Pulse spin to collect the pyruvate solution.
- NOTE:** The concentration of the pyruvate solution will be 400 mM
- C.6** Add 12.5  $\mu$ l pyruvate solution to each well of the Smart Reagent Plate containing the smart reagent solutions.

**NOTE:** If a quantitative method is ultimately used to measure activity (measuring the change in A490 with a plate reader), the reactions in this step and Step C.7 must be assembled on ice.



**C.7** Take 10  $\mu$ l of each enzyme solution from the Enzyme Lysate Plate and add to the corresponding well in the Smart Reagent Plate (e.g., B3 of the enzyme plate to B3 of the reagent plate, etc.) containing the pyruvate/reagent mixture.

NOTE: If a quantitative method is ultimately used to measure activity (measuring the change in A490 with a plate reader), the reactions in this step must be assembled on ice.

**C.8** Incubate the reactions at 30°C for 16 hours.

NOTE: If the activity of each enzyme is to be estimated by measuring the change in A490, use a 96-well plate reader to measure the A490 of each reaction at time zero immediately.

**C.9** After incubation, a color change from yellow to brown/orange should be observed when enzymes are fully active. Each reaction will gradually turn dark yellow, orange, or deep red to the naked eye if the corresponding transaminase is active. Absorbance at 490 nm is typically >0.25.

## END OF WORKFLOW



## STEPS

## TRANSAMINASE ACTIVITY PROTOCOL

The following protocol provides the steps to run an experiment for assessing transaminase activity. The steps detailed below are a starting point and further optimizations may be required.

Please see the section titled "Planning a Screening Experiment" or the appendix for additional details.

### Consumables and Reagents

- Clear flat-bottom 96-well microtiter plate
- Amine donor: 2-(4-nitrophenyl)ethan-1-amine hydrochloride (CAS, 29968-78-3) or other amine donor of choice
- Amine acceptor: Sodium pyruvate (CAS, 113-24-6) or other amine acceptor of choice
- Buffer: 0.5 M Potassium phosphate buffer (pH 7.4) or other buffer of choice
- Cofactor: pyridoxal 5-phosphate (PLP), monohydrate (CAS, 41468-25-1)
- Molecular biology grade water
- Optional: Substrate solvent of choice (e.g., DMSO, see Optimizing Reaction Conditions section in the appendix)

### Before You Begin

- Take out an enzyme plate from a -20°C freezer and pulse spin at 1500 xg for 1 minute to collect lysates. Let the plate equilibrate to room temperature for 30 minutes.

## WORKFLOW

**1** Prepare a 25 mM amine donor master mix as indicated below.

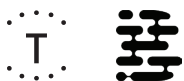
REAGENT	MASS OR VOLUME PER REACTION*	MASS OR VOLUME PER PLATE
2-(4-nitrophenyl)ethan-1-amine hydrochloride or other amine donor of choice	4.4 µmol	250 µmol
Pyridoxal 5-phosphate monohydrate (PLP)	48 µg (0.18 µmol)	2.7 mg (10 µmol)
0.5 M Potassium phosphate buffer, pH 7.4 or other buffer of choice	35.5 µl	2 ml
Molecular biology grade water	142 µl	8 ml

*\*Create a master mix for multiple reactions*

**2** Prepare a 400 mM amine acceptor master mix as indicated below.

REAGENT	MASS OR VOLUME PER REACTION*	MASS OR VOLUME PER PLATE
Sodium pyruvate or other amine acceptor of choice	5 µmol	400 µmol
Molecular biology grade water	12.5 µl	1 ml

*\*Create a master mix for multiple reactions*



**3** Prepare enzyme solutions by adding 100  $\mu\text{l}$  molecular biology grade water to each well in the enzyme plate. Seal and shake the plate at room temperature for 5 minutes with 1000 rpm agitation using a microplate shaker.

**4** Centrifuge the plate at 1500  $\times g$  for 1 minute to collect the enzyme solutions and then place the enzyme plate on ice.

**5** For each enzyme activity reaction, add the following reagents to a well of a 96-well plate according to the order listed below (top to bottom):

REAGENT	VOLUME PER REACTION
25 mM amine donor master mix	177.5 $\mu\text{l}$
400 mM amine acceptor master mix	12.5 $\mu\text{l}$
Enzyme solution (taken from a well of the enzyme plate)	10 $\mu\text{l}$
<b>Total</b>	<b>200 <math>\mu\text{l}</math></b>

NOTE: The remaining 90  $\mu\text{l}$  of enzyme solution for each enzyme can be used in additional reactions as necessary. Otherwise, store at 4°C with a sealed film for up to 5 days.

**6** Incubate the reaction mixture(s) at 30°C overnight for 16 hours.

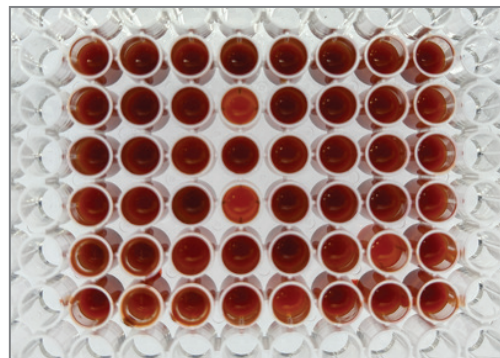
**7** Conduct color change assessments and/or absorbance measurements of choice.

## END OF WORKFLOW

## APPENDIX

### EXPECTED RESULTS

Positive reactions should exhibit a dark brown/orange color after a 16-hour incubation at 30°C. The image right is an example using a substrate that has activity with all enzymes.



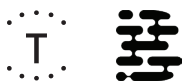
### TROUBLESHOOTING

OBSERVATION	TROUBLESHOOTING
<b>Substrate solubility in aqueous media is a challenge and the reaction mixture is cloudy</b>	Cloudiness in the reaction mixture, indicating incomplete substrate solubility, is typically acceptable and does not usually impede the reaction. While it is best to add the substrate fully solubilized in an aqueous buffer, substrates can alternatively be solubilized in 10% v/v DMSO or similar solvents such as short-chain alcohols, DMF, THF, and acetonitrile to ensure even concentration across all reactions. Furthermore, increasing the reaction temperature to 40–45°C can enhance substrate solubility. Refer to the Enzyme Typical Parameters table for the typical conditions tolerated by each enzyme. Additionally, increasing the pH might aid solubility, although this varies depending on the substrate. We advise against decreasing pH below 7.0.
<b>Low or no activity is found</b>	Try allowing the reaction to run for a longer time, increasing the temperature (to 40°C), increasing substrate concentration, and/or increasing the enzyme concentration. You can also run a control reaction using pyruvic acid as an amine acceptor to verify your reaction solution. Please refer to the control activity protocol for further details.  If issues persist, consider consulting the Optimizing Reaction Conditions section or contacting Twist Bioscience and bitBiome for further options, including enzyme refinement services for engineering enzymes with high activity.
<b>There are many hits and differences among them cannot be easily determined</b>	Consider stopping the reaction earlier or repeating the screen using a lower enzyme loading. If repeating, maintain DMSO concentration at <10% v/v.

### OPTIMIZING REACTION CONDITIONS

It's crucial to comprehend the inherent nature of both the substrate and product, as these are properties the enzyme cannot alter (e.g., solubility, pH, and temperature stability). Additionally, it's important to avoid conditions that may promote side reactions, lead to downstream issues, or are incompatible with the substrate or product. Evaluate the reaction performance at different pH levels (recommended: pH 7.4, 9, and 10; using potassium phosphate buffer at pH 7.4 and sodium borate at pH 9 and 10). Once an optimal pH is determined, investigate the effect of temperature on reaction performance (recommended: 30–55°C). Furthermore, consider evaluating the reaction at higher substrate loadings to optimize performance. If the substrate is not soluble in the reaction mixture, it may lead to substrate mass transfer limitation. In such cases, consider investigating the addition of a solubilizing co-solvent. Refer to the Enzyme Typical Parameters table for solvent concentration tolerance. Besides DMSO, other solvents such as short-chain alcohols, DMF, THF, and acetonitrile can also be explored.





## FAQ FOR QUALITY CONTROL OF ENZYME KIT MANUFACTURING

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### 1. HOW DO YOU ENSURE EACH ENZYME IN THE KIT HAS ADEQUATE ACTIVITY?

We check the activity of each enzyme in every batch with a standard colorimetric method that uses a plate reader to measure the change in absorbance at 490 nm after 16 hours of incubation at 30°C. All enzymes must show a significant change in A490 values in the test plate for each batch before they are packaged. This ensures that each enzyme meets our activity standards and performs reliably in your applications.

### 2. HOW OFTEN ARE QUALITY CONTROL CHECKS PERFORMED?

Quality control checks are performed after each batch of enzyme kits is manufactured. This ensures consistency, reliability, and high quality in all our products.

### 3. WHAT HAPPENS IF A BATCH DOES NOT MEET THE QUALITY STANDARDS?

A batch is not released for sale if it does not meet our stringent quality standards. It undergoes further analysis and corrective actions are taken to address any issues before it can be reconsidered for distribution.

### 4. CAN CUSTOMERS REQUEST QUALITY CONTROL REPORTS FOR THEIR SPECIFIC ENZYME KIT BATCH?

Yes, customers can request detailed quality control reports for their specific batch. These reports will include the A490 data from our standard colorimetric assay and the average mass quantity of each batch.