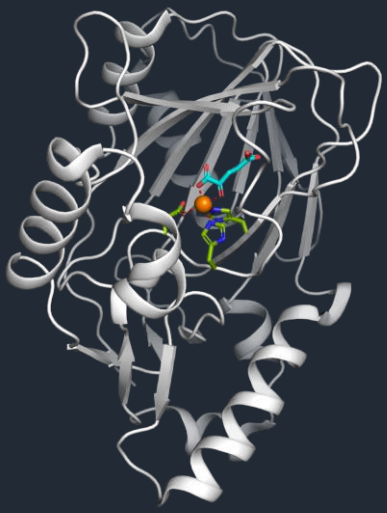
Manual

KGO Enzyme Panel
AminoAcid

Fe(II)/α-ketoglutarate-dep. oxygenases



Enzyme panel of KGOs for
Oxyfunctionalization

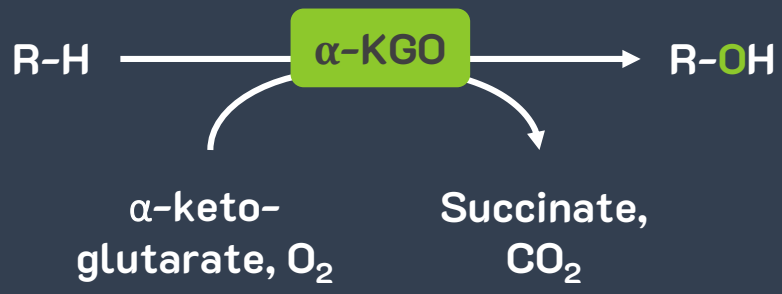
AMINOVERSE
EXPLORING ENZYME INNOVATION

Content

1 Introduction

Fe(II)- and α-ketoglutarate dependent oxygenases (KGOs) are non-heme iron proteins, that rely on α-ketoglutarate as a cofactor. These enzymes catalyse a variety of interesting reactions including, among others, hydroxylation, epoxidation, demethylation, ring formation or ring expansion.

α-KGOs are ideal biocatalysts for chemically challenging reactions



2 KGO Enzyme Panel

The KGO Enzyme Panel contains wild-type KGOs recombinantly produced in *Escherichia coli.*

The practical MTP format allows parallel screening of the entire KGO Enzyme Panel, followed by your GC, HPLC or MS analysis of choice.

Any KGO from the KGO Enzyme Panel yielding the desired product can be engineered towards even greater performance.

3 AminoAcid – KGO Enzyme Panel

The AminoAcid KGO Enzyme Panel contains computationally curated wild-type KGOs recombinantly produced in *Escherichia coli* that act on amino acids such as the following:

- L-Leucine
- L-Proline
- ...

Why curate?
Due to the inherently higher selectivity of KGOs compared to UPOs for example, the curation reduces overall panel size and thus screening efforts while simultaneously increasing chances to identify a hit.

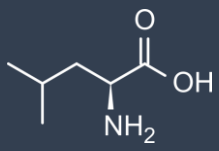
Aminoverse also offers 3 other curated KGO Enzyme Panels focused on the modification of different metabolites:

1. BioCarbon — fatty acids, terpenes, steroids
2. XNA — nucleic acids, nucleosides and –tides
3. Eureka — discovery panel with KGOs not fitting into the other three panels

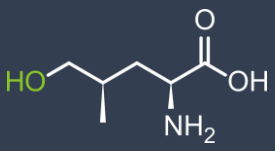
Contact us for more information!
enzymes@aminoverse.com

4 Example reactions

Examples for amino acid hydroxylation



L-Leucine



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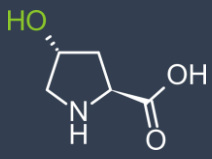
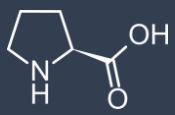
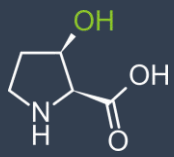
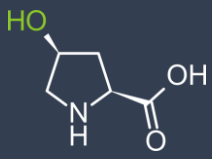






L-Proline



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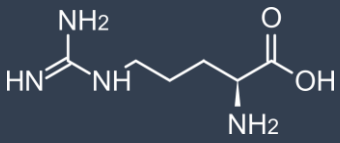
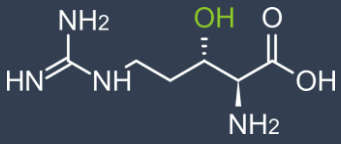


L-Arginine



[3]

[1] P. Lukat et al., *Chem. Sci.* **2017**, *8*, 7521–7527
[2] T. J. Smart et al., *Bioorg. Chem.* **2020**, *94*
[3] X. Yin, T. Zabriskie, ChemBioChem **2004**, 5, 1274–1277

5 Components and storage

KGO Enzyme Panel

- 1x MTP, contains lyophilized *E. coli* cell lysate supernatant + negative control (see plate layout on p. 9)
- Store at -20 °C
- Use directly after resuspension
- Caution: avoid repeated freeze-thaw cycles of the KGO Enzyme Panel

6 Plate content

The KGO Enzyme Panel MTP contains:

- 42 KGOs in duplicates
- Negative control in duplicates (well C6, C12)
- Extra KGO 11 in duplicates (well D6, D12), which can be used as positive control for test reactions to get familiar with the handling and the reaction before the actual screening

Reactions possible with 1 KGO Enzyme Panel:

- If you follow the recommended reaction protocol you can perform 1x 200 µL reactions per KGO

7 Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	KGO 28	KGO 49	KGO 75	KGO 88	KGO 100	KGO 246	KGO 28	KGO 49	KGO 75	KGO 88	KGO 100	KGO 246
B	KGO 30	KGO 50	KGO 77	KGO 90	KGO 102	KGO 247	KGO 30	KGO 50	KGO 77	KGO 90	KGO 102	KGO 247
C	KGO 32	KGO 57	KGO 78	KGO 93	KGO 131	neg. c. (no KGO)	KGO 32	KGO 57	KGO 78	KGO 93	KGO 131	neg. c. (no KGO)
D	KGO 34	KGO 60	KGO 80	KGO 94	KGO 146	KGO 11	KGO 34	KGO 60	KGO 80	KGO 94	KGO 146	KGO 11
E	KGO 40	KGO 61	KGO 81	KGO 95	KGO 217		KGO 40	KGO 61	KGO 81	KGO 95	KGO 217	
F	KGO 41	KGO 66	KGO 84	KGO 96	KGO 218		KGO 41	KGO 66	KGO 84	KGO 96	KGO 218	
G	KGO 45	KGO 69	KGO 85	KGO 98	KGO 221		KGO 45	KGO 69	KGO 85	KGO 98	KGO 221	
H	KGO 47	KGO 74	KGO 87	KGO 99	KGO 222		KGO 47	KGO 74	KGO 87	KGO 99	KGO 222	

8 Required reagents and equipment

- (Multi-channel) micro pipette
- dH_2O
- Buffer (*e.g.* 1000 mM KP_i buffer, pH 6.3)
- Sodium ketoglutarate
- Sodium ascorbate
- $FeSO_4$
- Substrate stock solution
- Reaction vessels, such as *e.g.*:
    - Microtiter plates
    - Reaction tubes
    - Glass vials
- Shaking incubator for 25 °C and 500 rpm

9 Preparation of reagents

- KGO Enzyme Preparation
  - Resuspend lyophilized KGO Enzyme Preparation/negative control with 115 µL dH_2O per well

- Buffer
  - Prepare buffer of choice, e.g. 1000 mM KP_i buffer, pH 6.3

- Sodium ketoglutarate stock
  - Prepare 100 mM stock in dH_2O
  - 0.9 mL* are sufficient for testing 42 KGOs plus negative control in 200 µL reactions

- Sodium ascorbate stock
  - Prepare 5 mM stock in dH_2O
  - 1.2 mL* are sufficient for testing 42 KGOs plus negative control in 200 µL reactions

Turn page for the rest of preparations

* Sufficient volume to accommodate for pipetting errors

- $FeSO_4$ stock
  - Prepare 50 mM stock in dH_2O
  - 1.2 mL* are sufficient for testing 42 KGOs plus negative control in 200 µL reactions

- Substrate stock
  - Prepare 50 mM substrate stock solution, *e.g.* dissolved in dH_2O
  - 1.2 mL* are sufficient for testing 42 KGOs plus negative control in 200 µL reactions

Note: Instead of adding the components individually, a master mix, containing KP_i buffer, sodium ketoglutarate, sodium ascorbate, $FeSO_4$ and substrate can be prepared and added to the resuspended KGOs accordingly.

* Sufficient volume to accommodate for pipetting errors

10 Protocol

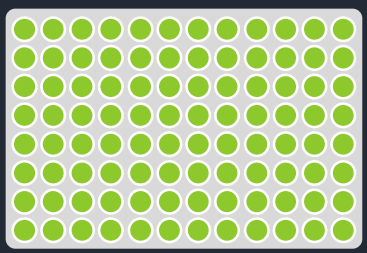
1. Centrifuge plate before opening it
2. Add **115 µL of resuspended KGO solution** or resuspended negative control to suitable reaction vessel, *e.g.* microtiter plate
3. Add **10 µL of 1000 mM KP_i buffer pH 6.3** to reaction vessel
4. Add **15 µL of 100 mM sodium ketoglutarate** stock solution
5. Add **20 µL of 5 mM sodium ascorbate** stock solution
6. Add **20 µL of 50 mM $FeSO_4$** stock solution
7. Add **20 µL of 50 mM substrate** stock solution to start the reaction
7. Incubate at **25 °C for 4 h* at 500 rpm**
8. Continue with your preferred extraction protocol, *e.g.* ethyl acetate extraction and drying over Na_2SO_4 to perform GC analysis

* Extending reaction time up to 24 h can lead to higher conversion rates

11 Pipetting scheme

Compound	Volume [µL]	Stock conc.	Final conc.
Resuspended KGO solution/ negative control	115	–	–
KP_i Buffer, pH 6.3*	10	1000 mM	50 mM
Sodium ketoglutarate*	15	100 mM	7.5 mM
Sodium ascorbate*	20	5 mM	0.5 mM
$FeSO_4$ x 7 H_2O*	20	50 mM	5 mM
Substrate solution*	20	50 mM	5 mM
Total volume	**200**		

➢ Reactions can be performed *e.g.* in microtiter plates or reaction tubes



* Note: Instead of adding the components individually, a master mix, containing KP_i buffer, sodium ketoglutarate, sodium ascorbate, $FeSO_4$ and substrate can be prepared and added to the resuspended KGOs accordingly.

12 Adapting the protocol

- Reaction volumes, reagent concentrations, and incubation times may be adapted to the desired reaction and the sensitivity of the detection method
- Reaction solutions can be analyzed using GC, HPLC or MS. Depending on the chosen analysis method, the extraction procedure may vary

Reaction volume

- Can be adjusted to specific needs; *e.g.* scaled up to 500 µL

Reaction time

- Can be varied according to specific needs and conversion of the substrate

Substrate stock solution and cosolvents

- Substrate concentration can be adjusted to specific needs and according to solubility

Feel free to contact us in case of questions or for tuning of the reaction conditions at enzymes@aminoverse.com.

13 Miscellaneous

The KGO Enzyme Panel is for *in vitro* research use only, not for diagnostic or therapeutic applications and has to be handled by qualified personnel.

The purchase of this product only authorizes the buyer to application of the KGO enzyme panel for internal research. Consult Aminoverse's Terms and Conditions for more information.

Reference information:
In publications, please refer to the KGOs of this enzyme panel by their *number* as "Aminoverse KGO*xxx*".

AMINOVERSE
EXPLORING ENZYME INNOVATION

Aminoverse solves enzyme challenges.
Founded in 2020, Aminoverse offers innovative enzyme products and services:

- Enzyme products and kits
  Ready-to-use enzymes for proof-of-concept studies up to commercial scale, *e.g.* the KGO Enzyme Panel or the UPO Enzyme Panel, and analyte detection kits, *e.g.* the Phosfinity series.

- CRO services
  Discovery of enzymes, enzyme feasibility studies, engineering of enzymes by Directed Evolution and machine learning, enzyme mutant library generation.

You have questions regarding this manual or about the application of the KGO enzyme panel?

We look forward to hearing from you.

Aminoverse B.V.
Daelderweg 9
6361HK Nuth
The Netherlands

enzymes@aminoverse.com
www.aminoverse.com

+31 4520 848 19

Aminoverse B.V.
Daelderweg 9
6361HK Nuth
The Netherlands

enzymes@aminoverse.com
www.aminoverse.com
+31 4520 848 19

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