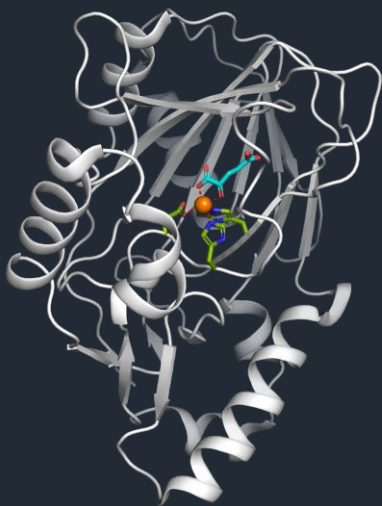


Manual

KGO Enzyme Panel

Eureka

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



Enzyme panel of KGOs for
Oxyfunctionalization

Content

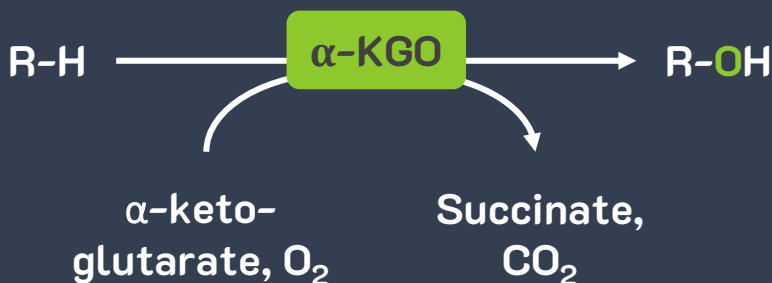
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Introduction

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



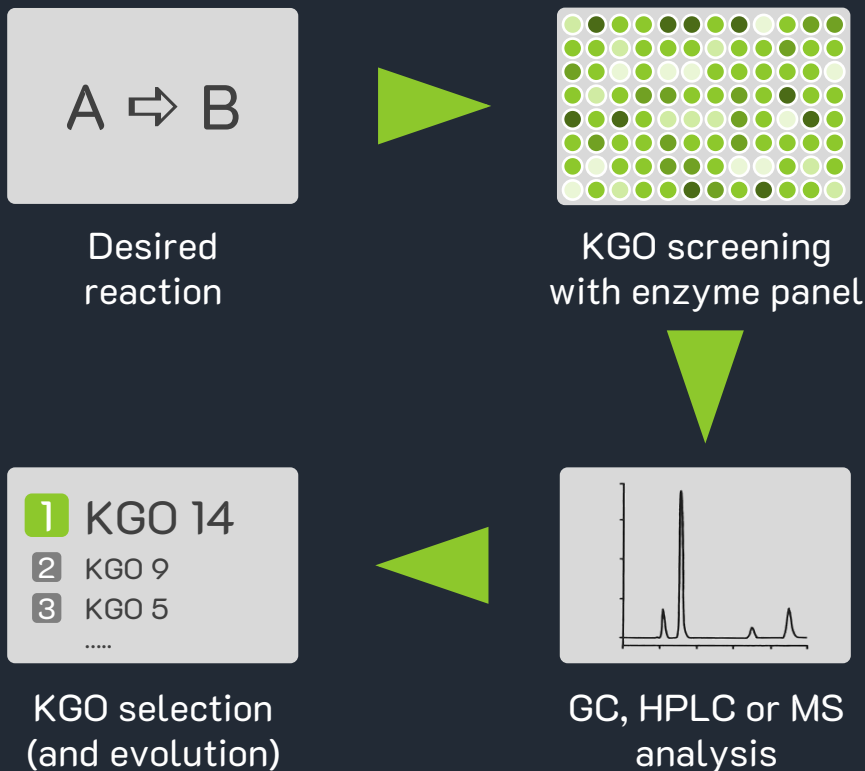
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KGO Enzyme Panel

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.



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Eureka – KGO Enzyme Panel

The Eureka KGO Enzyme Panel contains **computationally curated** wild-type KGOs recombinantly produced in *Escherichia coli* that were mined with focus on diversity. Therefore, these KGOs could act on a variety of compounds.

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:

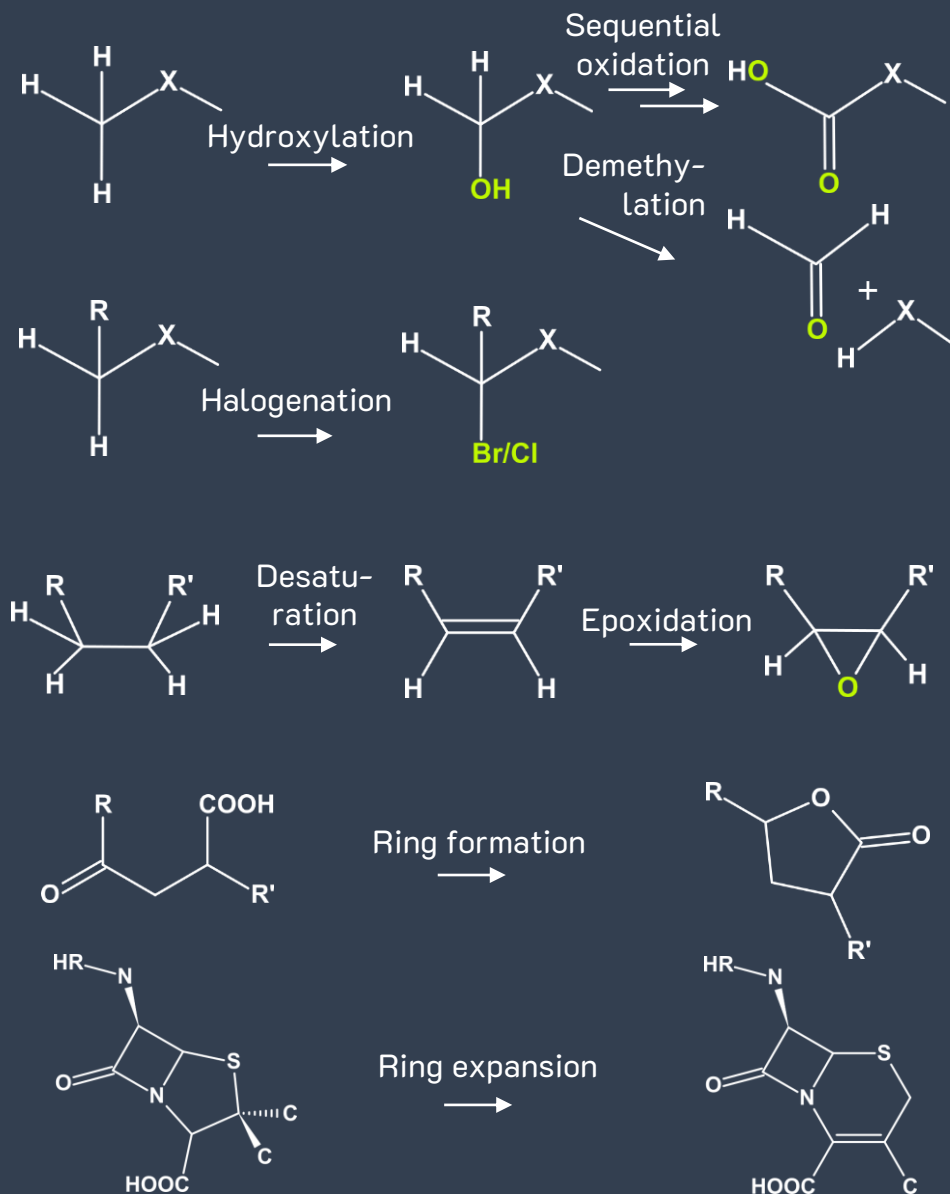
1. **BioCarbon** fatty acids, terpenes, steroids
2. **AminoAcid** amino acid modification
3. **XNA** nucleic acids, nucleosides and –tides

Contact us for more information!
enzymes@aminoverse.com

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Example reactions

Examples for the reaction scope of KGOs



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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



The KGO Enzyme Panel MTP contains:

- 41 KGOs in duplicates
- Negative control in duplicates (wells B6, B12)
- Extra KGO 11 in duplicates (wells C6, C12), 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:

- Following the recommended reaction protocol, the plate provides for performance of 2x 200 μ L reactions per KGO

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Plate layout

1 2 3 4 5 6 7 8 9 10 11 12

A	KG0 3	KG0 174	KG0 185	KG0 199	KG0 214	KG0 248	KG0 3	KG0 174	KG0 185	KG0 199	KG0 214	KG0 248
B	KG0 25	KG0 175	KG0 187	KG0 204	KG0 223	neg. c. (no KG0)	KG0 25	KG0 175	KG0 187	KG0 204	KG0 223	neg. c. (no KG0)
C	KG0 26	KG0 176	KG0 188	KG0 205	KG0 225	KG0 11	KG0 26	KG0 176	KG0 188	KG0 205	KG0 225	KG0 11
D	KG0 84	KG0 178	KG0 189	KG0 206	KG0 227		KG0 84	KG0 178	KG0 189	KG0 206	KG0 227	
E	KG0 168	KG0 180	KG0 191	KG0 209	KG0 234		KG0 168	KG0 180	KG0 191	KG0 209	KG0 234	
F	KG0 171	KG0 181	KG0 194	KG0 211	KG0 239		KG0 171	KG0 181	KG0 194	KG0 211	KG0 239	
G	KG0 172	KG0 183	KG0 197	KG0 212	KG0 240		KG0 172	KG0 183	KG0 197	KG0 212	KG0 240	
H	KG0 173	KG0 184	KG0 198	KG0 213	KG0 241		KG0 173	KG0 184	KG0 198	KG0 213	KG0 241	

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Required reagents and equipment

- (Multi-channel) micro pipette
- dH₂O
- Buffer (*e.g.* 1000 mM KP_i buffer, pH 6.3)
- Sodium ketoglutarate
- Sodium ascorbate
- FeSO₄
- Substrate stock solution
- Reaction vessels, such as *e.g.*:
 - Microtiter plates
 - Reaction tubes
 - Glass vials
- Shaking incubator for incubation at 25 °C and 500 rpm

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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 41 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 41 KGOs plus negative control in 200 μL reactions

Turn page for the rest of preparations



* Sufficient volume to accommodate for pipetting errors

- **FeSO₄ stock**
 - Prepare 50 mM stock in dH₂O
 - 1.2 mL* are sufficient for testing 41 KGOs plus negative control in 200 µL reactions
- **Substrate stock**
 - Prepare 50 mM substrate stock solution, *e.g.* dissolved in dH₂O
 - 1.2 mL* are sufficient for testing 41 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, FeSO₄ and substrate can be prepared and added to the resuspended KGOs accordingly.

* Sufficient volume to accommodate for pipetting errors

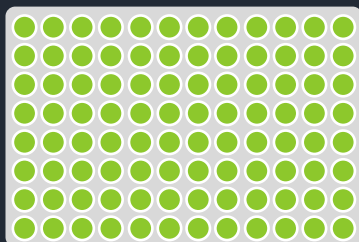
10 Protocol

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 KPi 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 $^\circ\text{C}$ 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

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 × 7 H ₂ O*	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, FeSO₄ and substrate can be prepared and added to the resuspended KGOs accordingly.

- 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.

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 KGOxxx”.



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

