

KMO – Kynurenine 3 – monooxygenase

Product specification

Acronym: KMO

Class: Enzyme

Origin: Human

Molecular weight: 55,8 kDa

Application: Screening & display technologies, Antibody development, Structural Biology.

Purity: >70%

Activity: Validated by spectrophotometric assay

Length: Full Length

TMD: 1

Biological function: Synthesis of quinolinic acid

Product description

KMO is required for synthesis of quinolinic acid, a neurotoxic NMDA receptor antagonist and potential endogenous inhibitor of NMDA receptor signaling in axonal targeting, synaptogenesis and apoptosis during brain development. Quinolinic acid may also affect NMDA receptor signaling in pancreatic beta cells, osteoblasts, myocardial cells, and the gastrointestinal tract.

KMO is a flavin adenine dinucleotide (FAD)-containing outer mitochondrial membrane enzyme of the Kyn pathway (KP). This pathway is a drug target of several pathological states, especially immunological diseases (e.g., cancer and chronic infection) and neurodegenerative and/or neuroinflammatory diseases (including huntington disease, Parkinson disease, and alzheimer's disease). It is initiated through the oxidative cleavage of l-tryptophan by indoleamine 2,3-dioxygenases, or tryptophan 2,3-dioxygenase to yield *N*-formyl-l-kynurenine (NFK). Subsequent hydrolysis by formamidase yields l-kynurenine (l-Kyn).

Protein Source: HuKMO wild type protein (Human KMO isoform 1)

Fig.1: AA sequence of hKMO protein

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10      20      30      40      50
NDSSVIGQRKK VAVIGGGGLVQ SLQACFLAKR NFQEDVYEAR EDTRVATFTR
60      70      80      90     100
GRSINLALSH RGRQALKWVQ LEDQIVSOGI PNRNFMHLS SGKKSALPYG
110     120     130     140     150
TKSQVILSYV RENVLNDLLT AAENYPMVKH HFNRLLKCN PEEGMDVLG
160     170     180     190     200
SRKVPKQVTC DI TVACDQAY STVRS-HIKK PRFDVYQQYT PAKYVFI TTP
210     220     230     240     250
PKNGDYVMEP NVLHINPRTN FNMIALPMMN KSFCTCLFMP FEEFEKLLTS
260     270     280     290     300
NDVVDFTQKY QDAIPLIGE KLLVQDFTLL TAQVNIQVKK SDFITKSKVY
310     320     330     340     350
LIRDAAHATV PFFRQGMVAQ FFDICVDFDI VNKFRNDI S CIEVFSRIAT
360     370     380     390     400
HDDHVLSDLS HYWYIEMVAH VNSSW-HQK VNRKFLKLI PSHFLPLVFN
410     420     430     440     450
VTFGRIRYIE AVQRNIMQKK VKNKGLFTLC DLCAISDSTV LDI YMGPMET
460     470     480
LIRPPANMT AHERNTTFP AKAVDSI EQT SNI TSR

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Affinity Tag: No affinity Tag on the protein and N-terminal His Tag.

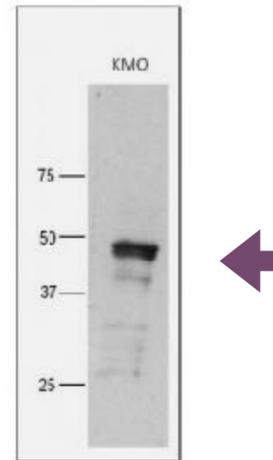
Production conditions: huKMO is expressed in a cell-free expression system, in the presence of lipid vesicles. 100 µg can be produced and qualified in about 1 week.

Quality analysis

Purity: Typically > 70% as determined by SDS-Page and Coomassie Blue staining.

Purification procedure: As standard, huKMO proteoliposomes are purified on a sucrose gradient. Further purification can be performed if required.

Fig.2: Proteoliposome hKMO after purification (Western Blot identification)



Assessment of functionality

Recombinant proteoliposomes containing huKMO have been shown to be active into liposomes. Its binding properties were validated using spectrophotometric experiments.

Cell-free expression systems provide a real alternative for membrane protein expression, enabling the study of structure and function of membrane proteins.

Methods:

The Human KMO protein was expressed in Synthelisis' cell-free system in the presence of liposomes to obtain proteoliposomes in one step reaction.

The reaction to hydrolysis L-kyn in L-3OH-Kyn is performed with NADPH: $L\text{-Kyn} + \text{NADPH} = L\text{-3OH-Kyn} + \text{NADP} + \text{H}_2\text{O}$. Spectrophotometric assay was used to characterize the NADPH disappearance, at 340nm.

Results :

The results show that recombinant KMO embedded in the lipid bilayer of the liposomes is correctly folded and oriented to enable the hydrolysis of L-kyn.

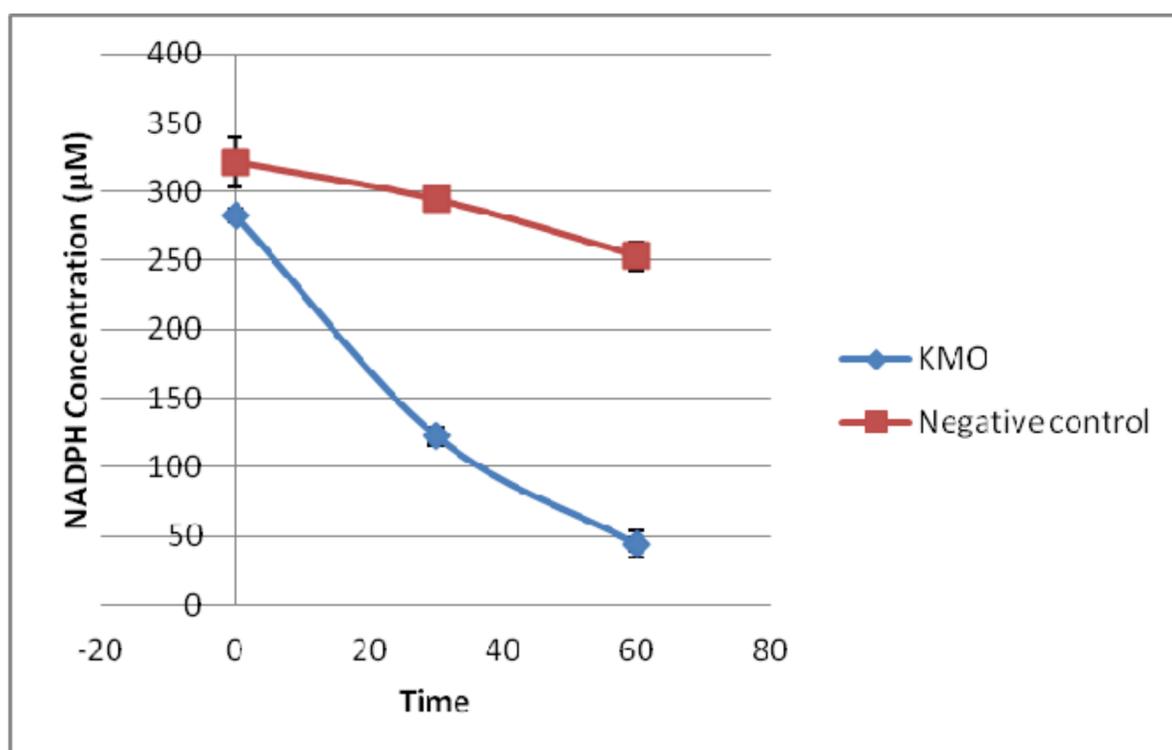


Fig.3: Kinetics of NADPH disappearance.

Formulation

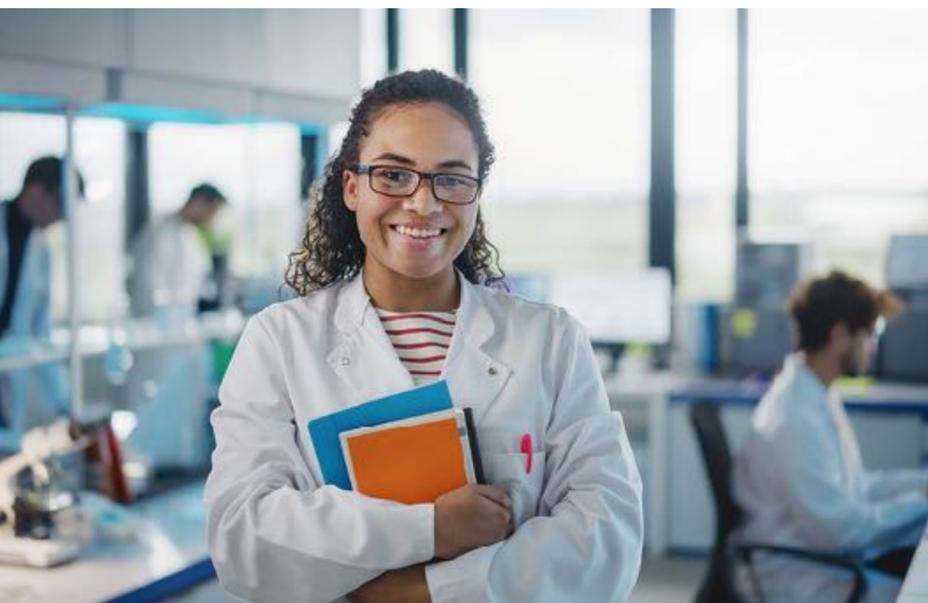
Buffer: Available in Tris 50mM, pH 7.5. Other buffers or customized formulation can be provided upon request.

Customized Hydrophobic matrix: Customized formulation with specific lipids like PEGylated or biotinylated lipids can be used upon request, as well as targeting molecules.

Storage/Stability: Store at +4°C for up to one week or several months at -80°C. Aliquot for storage. Do not freeze-thaw after aliquoting.

Use restrictions: For life science research use only.

Available sizes: 10µg, 20µg, 100 µg, 200 µg, 500 µg, bulk



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