**DESCRIPTION**

**Source**  
*E. coli*-derived  
Met1-Leu425, with an N-terminal Gly-Arg-Ala-His  
Accession # Q8N5Z0

**N-terminal Sequence Analysis**  
Gly

**Predicted Molecular Mass**  
48 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
40-42 kDa, reducing conditions

**Activity**  
Measured by its ability to form Kynurenic Acid from Kynurenine and alpha-Ketoglutarate.  
The specific activity is >170 pmol/min/μg, as measured under the described conditions.

**Endotoxin Level**  
<1.0 EU per 1 μg of the protein by the LAL method.

**Purity**  
>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

**Formulation**  
Supplied as a 0.2 μm filtered solution in HEPES and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- Assay Buffer: 20 mM HEPES, 50 mM NaCl, pH 8.0
- Recombinant Human α-Aminoadipate Aminotransferase (rhAADAT) (Catalog # 7927-AT)
- L-Kynurenine (Sigma, Catalog # K8625), 40 mM stock in 80 mM HEPES, 8 mM HCl, pH 8.0
- α-Ketoglutarate (Sigma, Catalog # K2010), 1 M stock in diH₂O
- Pyridoxal 5'-phosphate (Sigma, Catalog # P9255), 100 mM stock in 1 M HEPES, pH 8.0
- KYNA Development Mixture: 2 M ZnCl₂, 2 M NaOAc in diH₂O
- Standard: Kynurenic acid (Catalog # 0223), 50 mM stock in 100 mM NaOH
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

**Assay**

1. Dilute 50 mM Kynurenic acid standard to 400 μM in Assay Buffer. This is the first point of the standard curve.
2. Perform six additional one-half serial dilutions in Assay Buffer. The standard curve has a range of 156-10,000 pmol per well.
3. Dilute rhAADAT to 5 μg/mL in Assay Buffer.
4. Prepare Substrate Component Solution containing 50 mM HEPES, 20 mM α-ketoglutarate, 80 μM Pyridoxal 5'-phosphate, pH 8.0.
5. Immediately before use, prepare Substrate Mixture by combining 25 μL of Kynurenine with 50 μL of Substrate Component Solution, per well assayed.
6. Load 25 μL of each standard curve dilution into empty wells of a black well plate.
7. Load 25 μL of dilute rhAADAT into empty wells. Include a Blank containing 25 μL of Assay Buffer. This will serve as both enzyme and standard curve blank.
8. Add 75 μL of Substrate Mixture to all wells used.
9. Cover the plate with a plate sealer and incubate at room temperature for 30 minutes.
10. Add 100 μL of KYNA Development Mixture to all wells used.
11. Read plate at excitation and emission wavelengths of 338 and 405 nm, respectively, in endpoint mode.
12. Calculate specific activity:

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\text{Specific Activity (pmol/min/μg) = } \frac{\text{Adjusted Kynurenic acid produced}^* (pmol)}{\text{Incubation time (min)} \times \text{amount of enzyme (μg)}}
\]

*Derived from the Kynurenic acid curve using linear fitting and adjusted for Blank.

**Final Assay Conditions**

- rhAADAT: 0.125 μg
- α-ketoglutarate: 10 mM
- Pyridoxal 5'-phosphate: 40 μM
- Kynurenine: 10 mM

**PREPARATION AND STORAGE**

**Shipping**  
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.
Kynurenine aminotransferase II (KAT-II), also known as α-Aminoadipate Aminotransferase (AADAT), catalyzes the PLP-dependent transamination of aminoadipate to α-oxoadipate in the catabolism of lysine in the liver and also is the primary brain enzyme catalyzing the transamination of kynurenine to kynurenic acid (KYNA) (1). KYNA is an endogenous antagonist of the N-methyl-D-aspartate (NMDA) receptors with weaker effects on kainite and alpha-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptors, the other two ionotropic glutamate receptors (2, 3). KYNA also acts upon alpha-7 nicotinic acetylcholine receptors (α7 nAChRs) and importantly may suppress the pre-synaptic release of glutamate to confer neuroprotective effects against NMDA-receptor mediated over-stimulation. Also, KYNA is an endogenous ligand of the orphaned G protein-coupled receptor 35 (GPR35), found primarily in immune cells, and may induce inositol phosphate production and Ca2+ mobilization (4). Elevated levels of KYNA have been implicated in Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, epilepsy, schizophrenia and cognitive impairment (3, 5).

References:

PRODUCT SPECIFIC NOTICES
Coomassie is a registered trademark of Imperial Chemical Industries Ltd.