



DDAH Activity Assay Kit (Colorimetric)

05/21

(Catalog # K2089-100; 100 assays; Store at -20 °C)

I. Introduction:

The dimethylarginine dimethylaminohydrolase (DDAH) family of enzymes metabolizes the endogenous nitric oxide synthase (NOS) inhibitors, asymmetric dimethylarginine (ADMA), and N^G-monomethyl-L-arginine (L-NMMA or MMA) and plays an important role in the homeostatic control of nitric oxide (NO). Altered NO biosynthesis has been implicated in the pathogenesis of cardiovascular disorders such as hypertension, atherosclerosis etc. ADMA and L-NMMA are the endogenous inhibitors of all NOS isoforms. More than 90 % of the endogenous ADMA is metabolized by DDAH enzymes thereby regulating the NO production. Two DDAH isoforms (DDAH1 and DDAH2) have been identified in mammals. DDAH1 is widely expressed in all tissues, especially in liver and kidney whereas DDAH2 is the predominant isoform in the vascular endothelium, which expresses endothelial NOS. DDAH expression and activity has been associated with endothelial dysfunction and NO production. Genetic variation in DDAH1 and DDAH2 genes have been significantly associated with serum ADMA levels. **BioVision's DDAH Activity Assay Kit** provides a rapid, specific, and easy method for measuring the total DDAH activity in various sample types. In this assay, DDAH converts the DDAH substrate into Citrulline, which in turn is converted into a series of intermediates that along with the developer mix generates a colored product measured at 466 nm. The kit is simple, easy to perform, sensitive and is high-throughput adaptable.



II. Application:

- Measurement of DDAH Activity in various sample types

III. Sample Types:

- Tissue homogenates, cell lysates

IV. Kit Contents:

| Components | K2089-100 | Cap Code | Part Number |
|-----------------------|-----------|----------|-------------|
| DDAH Assay Buffer | 25 ml | NM | K2089-100-1 |
| DDAH Reagent A | 12 ml | Blue | K2089-100-2 |
| DDAH Reagent B | 5 ml | Amber | K2089-100-3 |
| DDAH Reagent C | 10 ml | NM | K2089-100-4 |
| DDAH Substrate | 100 µl | White | K2089-100-5 |
| Citrulline Standard | 1 vial | Yellow | K2089-100-6 |
| DDAH Positive Control | 100 µl | Green | K2089-100-7 |
| Plate Sealing Film | 2 | -- | K2089-100-8 |

V. User Supplied Reagents and Equipment:

- (NH₄)₂SO₄
- 1.5 ml centrifuge tubes
- Potter-Elvehjem glass homogenizer or Dounce Tissue Homogenizer (BioVision Cat# 1998)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge all vials prior to opening. Read the protocol before performing the assay.

- **DDAH Assay Buffer:** Warm to room temperature (RT) before use. Store at -20 °C.
- **DDAH reagent A and DDAH Reagent C:** Ready to use as supplied. Store at RT.
- **DDAH Reagent B:** Ready to use as supplied. Store at 4 °C.
- **DDAH Substrate:** Store at -20 °C, protected from light. Mix 10 µl of DDAH Substrate with 90 µl of DPPH Assay Buffer to prepare the DDAH Substrate working solution.
- **Citrulline Standard:** Reconstitute the vial in 100 µl of dH₂O to prepare 100 mM Citrulline Standard stock solution. Dilute the 100 mM Citrulline Standard stock to 500 µM Citrulline Standard by mixing 5 µl of the 100 mM Citrulline Standard stock with 995 µl of dH₂O. Store the 100 mM Citrulline Standard stock solution at -20 °C.
- **DDAH Positive Control:** Ready to use as supplied. Divide into aliquots and store at -20 °C.

VII. DDAH Activity Assay Protocol:

- DDAH Enzyme Sample Preparation: For tissues:** Homogenize 100 mg of tissues in 1 ml DPPH Assay Buffer in a Potter-Elvehjem glass homogenizer at 4 °C. Centrifuge at 14,000 x g for 20 min at 4 °C and collect the clear supernatant. Add 400 mg of (NH₄)₂SO₄ to the clear supernatant and keep the solution on ice for 30 min. Centrifuge at 14,000 x g for 10 min at 4 °C and collect the pellets. Dissolve the pellets in 0.3-0.5 ml DPPH Assay Buffer and determine the protein concentration using BCA Protein Assay (BioVision Cat# K819). Adjust the protein concentration of the DDAH enzyme sample(s) to 20 mg/ml for the assay.
- DDAH Substrate Addition:** For each sample, prepare three parallel 1.5 ml centrifuge tubes labeled as **Sample**, **Spiked Sample** and **Sample Background**. To the **Sample** tube, add 10-40 µl of **DDAH enzyme sample** and 5 µl DDAH Substrate working solution, and adjust the volume to 50 µl with DDAH Assay Buffer. To the **Spiked Sample tube**, add the same 10-40 µl of **DDAH enzyme sample**, 5

