



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.

DDAH	DDAH Reagent A	ı	Developer Mix	
DDAH Substrate -	Citrulline	Intermediate -		Colored Product (OD = 466 nm)

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:

- 1. 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.
- 2. 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

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 μ I DDAH Substrate working solution and 4 μ I of 500 μ M Citrulline Standard (i.e. 2 nmol), and adjust the volume to 50 μ I with DDAH Assay Buffer. To the **Sample Background** tube, add the same 10-40 μ I of **DDAH enzyme sample**, and adjust the volume to 50 μ I with DDAH Assay Buffer. See the table below.

	<u>Sample</u>	Spiked Sample	Sample Background
DDAH enzyme sample	10-40 µl	10-40 µl	10-40 µl
DDAH substrate working solution	5 µl	5 µl	
Citrulline Standard (500 µM)		4 µl	
Adjust volume using DDAH Assay Buffer to	50 µl	50 µl	50 µl

To the **Positive Control** tube, add 5-10 μ I of DDAH Positive Control, 5 μ I DDAH Substrate working solution, and adjust the volume to 50 μ I with DDAH Assay buffer. Incubate all the tubes including Sample, Spiked Sample, Sample Background and Positive Control for exactly 45 min at 37 °C. Stop the reaction by adding 50 μ I of DDAH Reagent A. Vortex and centrifuge the tubes at 3000 x g for 10 min. Transfer 90 μ I of the supernatant from the Sample, Spiked Sample Background, Positive Control tubes into wells of a 96-well clear plate.

3. Developer Mix Addition: Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Developer Mix by mixing one part of DPPH Reagent A with two parts of DPPH Reagent B.

	<u>Developer Mix</u>
DDAH Reagent B	34 µl
DDAH Reagent C	68 µl

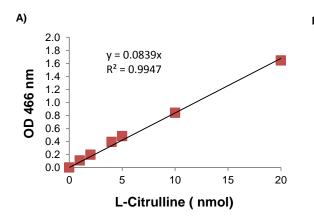
Add the Developer Mix to all wells including Sample, Spiked Sample, Sample Background Control, Standard and Positive Control wells. Cover the plate with a Plate Sealing Film and place it on a plate shaker for 1 min. Incubate the plate at 100 °C heat block for 60 min, protected from light. Cool the plate on ice for 10 min.

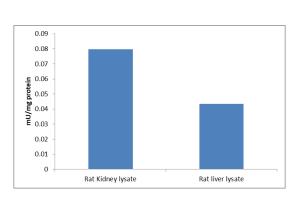
- 4. Measurement: Measure the absorbance in a microplate reader at 466 nm at RT.
- 5. Calculation: Determine DDAH activity in the sample (s) using the following equations:

Where: 2 is the spiked Citrulline Standard amount (nmol)

T is the enzyme reaction time (45 min)

C is the amount of protein per reaction (mg)





Figures: A) Citrulline Standard Curve. B) Estimation of DDAH activities in rat liver and kidney lysates. Sample preparation and assays were performed following the kit protocol.

VIII. Related Products:

Nitric Oxide Synthase Activity Assay Kit (C) (K205) EZCell™ Intracellular Nitric Oxide Synthase Detection Kit (K207) Nitric Oxide Fluorometric Assay Kit (K252) Nitrite Assay Kit (Griess Reagent) (K544) Nitric Oxide Synthase Activity Assay Kit (F) (K206) Nitric Oxide Synthase Inhibitor Screening Kit (F) (K208) Nitric Oxide Colorimetric Assay Kit (K262) Nitric Oxide Cell-Based HTS Assay Kit (K979)

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