ab308278 – DDAH Activity Assay Kit (Colorimetric)

Measurement of DDAH Activity in various sample types
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
DDAH Assay Buffer	25 ml	-20 °C
DDAH Reagent A	12 ml	-20°C
DDAH Reagent B (Avoid light)	5 ml	-20°C
DDAH Reagent C	10 ml	-20°C
DDAH Substrate	100 µl	20 °C
Citrulline Standard	1 vial	-20 °C
DDAH Positive Control	100 µl	-20 °C
Plate Sealing Film	2	---

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

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 $(NH_4)_2SO_4$ 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. 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 µl DDAH Substrate working solution and 4 µl of 500 µM Citrulline Standard (i.e., 2 nmol), and adjust the volume to 50 µl with DDAH Assay Buffer. To the Sample Background tube, add the same 10-40 µl of DDAH enzyme sample, and adjust the volume to 50 µl with DDAH Assay Buffer. See the table below.

	Sample	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 µl of DDAH Positive Control, 5 µl DDAH Substrate working solution, and adjust the volume to 50 µl 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 µl of DDAH Reagent A. Vortex and centrifuge the tubes at 3000 x g for 10 min. Transfer 90 µl of the supernatant from the Sample, Spiked Sample, Sample Background, Positive Control tubes into wells of a 96-well clear plate.

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.

	Developer Mix
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.

Measurement:

Measure the absorbance in a microplate reader at 466 nm at RT.

Calculation:

Determine DDAH activity in the sample (s) using the following equations:

$$\text{DDAH (mU/mg)} = (OD_{Sample} - OD_{SBC}) / (OD_{(Spiked\ Sample)} - OD_{Sample}) \times 2 \times T \times C\ \text{(nmol/min*mg)}$$

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)

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)