FT-39423B

ADHP

A highly sensitive, specific and stable fluorogenic substrate for peroxidase

Product Information

Name :	**ADHP (10-Acetyl-3,7-dihydroxyphenoxazine)** [10H-Phenoxazine-3,7-diol, 10-acetyl-, N-Acetyl-3,7-dihydroxyphenoxazine]			
Catalog Number :	FP-39423A,	5 mg	FP-39423F,	50 mg
	FP-39423B,	10 mg	FP-39423H,	500 mg
	FP-39423C,	10 x 10 mg	FP-39423I,	1 g
	FP-39423D,	25 mg		
Structure :	$C_{14}H_{11}NO_4$ – CAS: 119171-73-2 – MW: 257.25			
Molecular Weight :	257.25			
Solubility:	DMSO, DMF or CH_3OH			
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (buffer pH 8) = 280 nm / none			
	$\lambda_{exc}\backslash\lambda_{em}$ (end product) = 550 / 585 nm			
EC (M^{-1} cm^{-1}) **:**	6100 / 64 000 (end product)			

Storage: Store at -20ºC Protect from air, light and moisture (M) .

Directions for use

Protocol 1 - Measurement of hydrogen peroxide

1. Prepare incubation medium:125 mM KCl, 20 mM HEPES, pH 7.0, 2 mM KH_2PO_4, 4 mM ATP, 5 mM $MgCl_2$, 1 µM ADHP, 5 U/ml horseradish peroxidase (HRP), and 20 U/ml Cu,ZnSOD, and maintain at 37°C.
2. Detect the change in the concentration of H_2O_2 in the medium as an increase in ADHP fluorescence using excitation and emission wavelengths of 550 and 585 nm, respectively.
3. Calibrate the response of ADHP to H_2O_2 either by sequential additions of known amounts of H_2O_2 or by continuous infusion of H_2O_2 at 100-1000 pmol/min.
4. Calculate the concentration of commercial 30% H_2O_2 solution from light absorbance at 240 nM using E^{240}mM = 43.6 cm^{-1}; dilute the stock solution to 100 µM with water and use for calibration immediately.

Other protocols can found in the literature.

Protocol 2 – Detection in Peroxide assays

1. Prepare a 10 mM stock solution of ADHP reagent with dimethylsulfoxide.
2. Prepare a 20 mM H_2O_2 solution in ELISA reaction buffer, usually PBS.
3. Prepare a working solution containing 50 µM ADHP reagent and 200 µM H_2O_2.

4. Add 100 µl of the working solution to each of the microtiter plate wells and incubate for 30 min at room temperature.
5. Detect peroxidase activity either fluorometrically of spectrophotometrically.

See also ADHP ELISA Assay kit #HS6240 in related products.

Additional information

ADHP (10-Acetyl-3,7-dihydroxyphenoxazine) is regarded as the best fluorogenic substrate for peroxidase because it is highly specific and stable. The substrate itself is nearly colorless and nonfluorescent until it is oxidized by H_2O_2 in the presence of horseradish peroxidase (HRP) to become the highly red fluorescent resorufin.
Excitation can be done at 530-571nm, and emission occurs at 590-600nm.

Related products

- Dimethylsulfoxide anhydrous, JW7390
- PBS, 68723A
- Hydrogen peroxide solution, JQ6550
- ADHP ELISA Peroxidase Assay kit, HS6241
- FCCP, respiratory uncoupler, 333864
- HorseRadish Peroxidase HRP, UP146500
- HEPES, UP061940
- ATP, 00064A
- Resorufin FP-95432B

References

- **Panopoulos A**, *et al*. *J Biol Chem* **280**, 2912-23 (2005)
- **DeYulia GJ Jr**, *et al*.. *Proc Natl Acad Sci U S A* **102**, 5044-9 (2005)
- **Velasco G** *et al. Am J Respir Cell Mol Biol* (2004)
- **Pastor I**, *et al. Anal Biochem* **334**, 335-43 (2004)

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06