FT-24119

Alkaline Phosphatase substrates

Product Information

Name :	DDAO phosphate 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate, diammonium salt		
Catalog Number :	<u>FP-73967A</u> , 5mg		
Structure :	$C_{15}H_{18}Cl_2N_3O_5P$		
Molecular Weight :	MW= 422.2		
Solubility:	Soluble in dH2O or DMF		
Absorption / Emission :	$\lambda_{\rm exc} \setminus \lambda_{\rm em} = 646/659 \text{ nm}$		
-			
Name :	FDP,		
	fluorescein diphosphate, tetraammonium salt		
Catalog Number :	<u>FP-72573A</u> , 5mg		
Structure :	$C_{20}H_{26}N_4O_{11}P_2$		
Molecular Weight :	MW= 560.39		
Solubility:	Soluble in pH 7-8 buffer (not phosphate buffer)		
Absorption / Emission :	$\lambda_{\rm exc} \setminus \lambda_{\rm em} = 490/514 \ {\rm nm}$		
Name :	MUP,4-methylumbelliferyl phosphate, free acid		
Catalog Number :	<u>FP-24119A</u> , 100mg		
Structure :	$C_{10}H_9O_6P$		
Molecular Weight :	MW= 256.13		
Solubility:	Soluble in DMSO or in pH 7-8 buffer (not phosphate buffer)		
Absorption / Emission :	$\lambda_{\rm exc} \lambda_{\rm em} = 360/449 \text{ nm}$		
Name :	DiFMUP, 6,8-difluoro-4-methylumbelliferyl phosphate		
Catalog Number :	<u>FP-58657A</u> , 5mg		
Structure :	$C_{10}H_7F_2O_6P$		
Molecular Weight :	MW= 292.1		
Solubility:	Soluble in DMSO		
Absorption / Emission :	$\lambda_{exc} \lambda_{em} = 358/455 \text{ nm}$		
Name :	MUP ,4-methylumbelliferyl phosphate, disodium salt		
Catalog Number :	<u>FP-30045A</u> , 100mg		
Structure :	C10H7Na2O6P		
Molecular Weight :	MW= 300.11		
Solubility:	Soluble in DMSO or water		
Absorption / Emission :	$\lambda_{\rm exc} \lambda_{\rm em} = 360/449 \text{ nm}$		
Storage: $-20^{\circ}C > 1$ yes	Storage: $-20^{\circ}C > 1$ year. (M) Protect from light and moisture		

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Introduction

MUP has been in widespread use in fluorescence-detected ELISAs for many years.3 In addition, Dr. Hans Tanke and co-workers have described a hybridization assay for PCR products with a detection limit of 0.15 femtomoles in which immunoenzymatic analysis using MUP was found to be advantageous among a number of nonisotopic detection formats in terms of dynamic range and simplicity of instrumentation.

MUP is fluorogenic substrate for phosphatases. It is widely used for detecting phosphatases in solution. However this phosphatase substrate is not well suited for living cell or continuous assays since MU (4methylumbelliferone), the enzymatic product, which only develops maximum fluorescence at pH value of >10. Thus it is also difficult to use MUP for the detection of phosphatases that have acidic optimal pH range such as acid phosphatases. As alternative, the MUP Plus can be used.

DiFMUP is a fluorinated 4-methylumbelliferyl phosphate (MUP) derivative that has a lower pKa than that of MUP, making DiFMUP an excellent substrate for continuously assaying acid phosphatases at low pH. The reaction product of DiFMUP has excitation/emission maxima of ~358/450 nm.

FDP is a colorless and nonfluorescent substrate for alkaline phosphatases. Sequential alkaline phosphatase mediated hydrolysis of its two phosphate substituents yields weakly fluorescent fluorescein monophosphate followed by strongly fluorescent fluorescein (excitation/emission ~490/514 nm). FDP is not very thermally stable, and special cautions need be excised for storing the solid sample and stock solutions.

DDAO contains only a single hydrolysissensitive moiety, thereby avoiding the biphasic kinetics of fluoresceinbased substrates. DDAO-phosphate yields a hydrolysis product that is efficiently excited by the 633 nm spectral line of the He-Ne laser to produce bright red fluorescence with absorption/emission maxima of ~646/659 nm. Although the substrate itself is fluorescent, the difference between the substrate's excitation maximum and that of the product is over 200 nm, allowing the two species to be easily distinguished. DDAO phosphate has good water solubility, a low KM and a high turnover rate.

Catalog product	substrate	detection
FP-73967A	DDAO	Protein phosphatase 2A (PP2A)
FP-72573A	FDP	Protein tyrosine phosphatases
		Alkaline phosphatase
FP-24119A	MUP	Alkaline phosphatase
FP-58657A	DiFMUP	Acid phosphatase
		Protein phosphatase 1 (PP1)
		Protein phosphatase 2A (PP2A)

Directions for use

Protocol may be found in the literature.

References

DDAO-Phosphate

- Jakimiuk A. *et al.*, Estrogen Receptor α and β Expression in Theca and Granulosa Cells from Women with Polycystic Ovary Syndrome, The Journal of Clinical Endocrinology & Metabolism Vol. 87, No. 12 5532-5538 (2002) <u>Article</u>
- Krebs J. et al., Activation of Membrane-associated Procaspase-3 Is Regulated by Bcl-2, J. Cell Biol., Volume 144, Number 5, 915-926 (1999) <u>Article</u>
- Munir I. *et al.*, Insulin Augmentation of 17α-Hydroxylase Activity Is Mediated by Phosphatidyl Inositol 3-Kinase But Not Extracellular Signal-Regulated Kinase-1/2 in Human Ovarian Theca Cells, *Endocrinology* Vol. 145, No. 1 175-183 (2004) <u>Article</u>
- Wei-Qing Wang, et al., « Kinetic and Mechanistic Studies of a Cell Cycle Protein Phosphatase Cdc14 », J. Biol. Chem., 279, 30459 (2004) <u>Article</u>
- Yen H-W, *et al.*, Selective alterations in insulin receptor substrates-1, -2 and -4 in theca but not granulosa cells from polycystic ovaries, *Molecular Human Reproduction*, Vol. 10, No. 7, pp. 473-479 (2004) <u>Article</u>

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FDP

- Lizana L. et al., Controlling the rates of biochemical reactions and signaling networks by shape and volume changes, PNAS, 105: 4099 - 4104 (2008) <u>Article</u>

- Lovett FA et al., Convergence of *Igf2* expression and adhesion signalling via RhoA and p38 MAPK enhances myogenic differentiation, *J. Cell Sci.*, 119: 4828 - 4840 (2006) Article

Related Products

- Goat anti-mouse IgG-Phosphatase alkaline, <u>437050</u>

- TBS Tween 20 buffer, FM2251
- PVDF membrane, <u>T07590</u>

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