



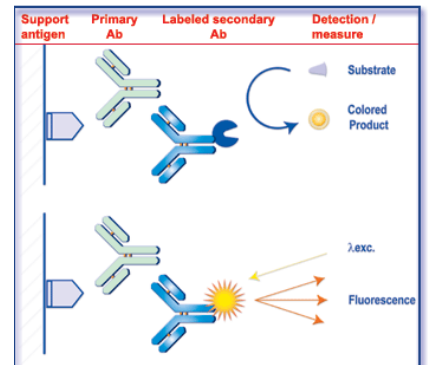
Enzymatic substrates for ImmunoAssays

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Introduction

Peroxidase (HRP) as well as Alkaline phosphatase (AP) conjugates are extensively used as secondary detection reagents in EnzymeImmunoAssays (EIA) such as ELISAs, immuno-histochemical techniques, Northern, Southern and Western blot analyses. While HRP has been more popular than AP thanks its flexibility in substrates and rather lower cost, it also has some drawbacks and limitations in some applications –as AP also has! See below the substrate selection guide-. Lab habits also drives for one or the other enzyme label.

Interchim provides a large choice of high quality substrates for EIAs by colorimetry, fluorimetry (FLISA) or luminometry (CLIA). Formulated substrates dedicated to specific techniques (ELISA, Blotting, MicroArray, ImmunoHistoChemistry and FlowCytometry) are described in detail in their corresponding section.



Selected substrates formulated products – by techniques

Technique (products description)	Chromogenic	Fluorescent	ChimioLuminescent
ELISA -HRP	TMB ELISA soln	FDP ELISA kit	UptiLight ECL ELISA substrate
ELISA –AP	pNPP ELISA soln AP-Red, AP-Blue		
ELISA –b-Gal	b-Galactoside kit		
Blotting - HRP	TMB blotting soln opDAB kit		UptiLight ECL Blotting substrate
Blotting - AP		BCIP/NBT soln	VisiGlo AP
IHC - HRP	opDAB kit		
IHC - AP			
IHC – b-Gal	MUF		

Selection guide for enzymatic substrates in immunodetections

Enzyme	Substrates	Applications	Pros	Cons
(HRP) Peroxidase			Rapid kinetic Numerous substrates available	Back ground in blood samples and some tissues Les staining stability than AP
	Chromogenic, soluble (TMB, ABTS, OPD...)	ELISA	Easy to use, popular	Sometimes light sensitive coloration
	Chromogenic, precipitating (CN, AEC, DAB,...)	WB, SB, IHC	Easy to use, popular	
	Fluorogenic (ADHP,...)	ELISA, MicroArray WB, SB, IHF		
	Chemiluminescent (Luminol,...UptiLight™)	ELISA WB, SB, IHC	High sensitivity High sensitivity	Need a luminometer Need a radiographic equipment,

				or a CDD camera, or a light scanner
(AP) Alkaline Phosphatase			<i>More sensitive and linear response</i>	<i>Often less economic than HRP</i> <i>High molecular weight conjugates</i>
	<u>AP Chromogenic, soluble (pNPP)</u>	ELISA	Linear kinetic Often more sensitive than HRP	
	<u>AP Chromogenic, precipitating (BCIP/NBT,...)</u>	WB, SB, IHC	Stability of staining	Back ground in some samples
	<u>Fluorogenic (MUP, FPD)</u>	ELISA, IHF	Sensitivity	Need fluorescence expensive equipment
	<u>Chemiluminescent (VisiGlo)</u>	ELISA, WB	High sensitivity	Need a luminometer, or an imager
Oxidases (β-galactosidase,...)				
	<u>Chromogenic substrates (X-Gal, ONG, MUG,...)</u>	GRA, IHC	High sensitivity	
	<u>Fluorogenic (MUG)</u>	GRA, IHC	High sensitivity	

WB: Western Blotting; SB: Southern Blotting; IF: Immuno(Histo)Fluorescence; IHC: ImmunoHistoChemistry; ELISA: Enzyme Linked Immunosorbent Assay; GRA: Gene Reporter Assay

Technical tip – Enzyme assays

Enzymes are powerful signal amplification systems in bioassays. Because of their ability to be conjugated with antibodies or other probes, while preserving their activity, peroxidase (HRP) and alkaline phosphatase (AP) are the most popular enzymatic markers in immunoassays. Substrates are converted in colored or light emitting products detected by spectrophotometry or other light detectors. Depending on the technique, color or light is measured either at the site where the enzyme was immobilized (precipitating substrates), or released in solution (soluble substrates, i.e. for ELISA). The intensity of the developed color or light emission should correlate to the concentration of the primary antibody or the respective antigen.

Technical tip – Fluorescence

Fluorescence is the generation of light upon excitation by a light of different (usually lower) wavelength. Benefits of this techniques include:

- low background, excepted from some samples especially in the visible range (autofluorescence)
- high signal and highly sensitive detection instrument
- different available excitation and emission allow multiplex

Technical tip – Chemiluminescence

(Bio)Chemiluminescence is the generation of electromagnetic radiation, i.e. visible light, by the release of energy from a (enzymatic) chemical reaction. Benefits of this techniques include:

- Most samples have no 'background' signal, i.e. they do not themselves emit light. No interfering signal limits sensitivity
- Avoid some limitations and difficulties known in fluorescence 1/ due to ratiometric Measurement 2/ due to scattering of the incident light to the detector, especially when samples are somewhat turbid.

As a result, CL provides highest sensitivity with robustness and easy to use in R&D and diagnostic bioassays.

Technical tip – Peroxidase (HRP)

Peroxidase (Raifort Horseradish Peroxidase) is one of the most commonly used enzymes for labeling, as it is cheap and versatile. Our peroxidase is selected for its high activity and conjugated to the antibodies following an optimized process, which results in highly sensitive and stable antibodies. HRP reacts with an extensive range of soluble and insoluble substrates (please refer section Enzymes-substrates), standards being TMB for colorimetry in ELISA and blotting, and UptiLight chemiluminescent substrate (BM4961) for higher sensitivity. One of the main problems associated to HRP is non-specific staining resulting from endogenous peroxidase activity in some immunocytochemistry applications.

Immunoperoxidase reagents, are not recommended for tissues or cells in which endogenous peroxidase activity is difficult to suppress. In such cases, other immunoenzyme reagents may be used. Alternatively, anti-horseradish peroxidase (HRP) antibodies conjugated with alkaline phosphatase or fluorophores, or complexed with colloidal gold particles may be used to enhance signal and reduce background from endogenous enzymes, since the final signal does not depend on the enzyme activity of peroxidase, but on the antigenicity of horseradish peroxidase.

Search secondary antibody peroxidase conjugates with [Ab2Search engine](#)

Technical tip – Alkaline Phosphatase label (AP)

Alkaline Phosphatase (AP) is an enzyme, which is isolated from calf intestines. It gives a more linear activity than peroxidase, and is suitable for most immunodetections. AP is recommended for applications where high levels of endogenous peroxidase are present and for quantitative and sensitive measurements (AP reaction rate is more linear than HRP, the sensitivity can be increased by just allowing the reaction to proceed for longer periods of time).

Recommended substrates for alkaline phosphatase are : BCIP/NBT for blotting and IHC applications (cat.# UP096051) and pNPP for ELISA (cat.# UP732500). The reaction with pNPP allows kinetic readings. Endogenous AP activity found in some samples can be inhibited by levamisole.

Technical tip –Glucose Oxidase (GO)

The glucose oxidase enzyme (GOx) (EC 1.1.3.4) catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone.

Glucose oxidase is rarely used in immunoassays, but more widely used in enzyme assays for biochemistry including biosensors in nanoT., beside diagnostic applications.

Please [check here GO products](#) .

Substrates for horseradish peroxidase

Peroxidase reacts with substrate H_2O_2 , which in turn reacts with a chromogenic substrate to give a colored end product. The final product is either soluble (for solid phase assays such as ELISA), or precipitates near immobilized peroxidase (positive sites), visualizing spots (Dot blot) or bands (Western Blotting). A large variety of chromogenes is available in powder (table below) and formulated (see following sections, by techniques).

HRP substrates	ref ^(a)	Type ^(b)	Comment
OPD tablets (15mg each)	270861, 50tabs	Chromo. purple	(o-Phenylene Diamine) has been the most popular chromogenic HRP substrate until less toxic and equally sensitive chromogens were proposed (TMB), as well as even more sensitive chemiluminescent ones (Luminol, UptiLight). Pre-weighed tablets offer a safer and more convenient use.
OPD, Ultrapure powder CAS:[95-54-5]; MW: 108.1	02673F, 25g 02673G, 50g	Chromo. Purple	
ABTS, Liquid substrate (ready to use)	UP732550 100 ml	Chromo green	(2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate) popular substrate for HRP, giving a soluble green end product measured at 450nm.
ABTS tablets (10mg each)	42387C, 50 tablets		
ABTS, Ultra pure grade powder CAS:[30931-67-0]; MW : 548.7	UP423876 1 g UP423877 10 g		
AEC, powder CAS:[132-32-1]; MW : 310.28	036310, 50g	Chromo red	(3-amino-9-ethylcarbazole) relatively less toxic nature compared to DAB. It produces a red colored end product at the positive sites, which gives a good contrast with blue hematoxylin counter stain, and is also useful for double labeling. It is less sensitive than DAB, but exhibits less background. Slightly more sensitive than 4-CN.
4-CN, powder CAS:[604-44-4]; MW : 178.62	UP007490, 25g UP007491, 25g	Chromo blue-black	(4-Chloro-1-Naphtol) yields an intense blue-black product in a easily controlled reaction. The least sensitive HRP substrate but exhibits less background than DAB
TMB, Ultrapure powder CAS:[64285-73-0]; MW 331.3	UP15426D, 1g UP15426E, 5g	Chromo blue/yellow	(3,3',5,5'-tetramethylbenzidine) has become the most popular chromogenic HRP substrate, notably in ELISA (works also in blotting and IHC). It produces a blue color, measured at 370nm or 652nm, or a yellow colour (when reaction is stopped by adding an acid), measured at 450nm. Sensitivity is greater than OPD and ABTS, and background is very low. Several derivatives exist (TMB-z...)
TMB, solution (Ready-to-use for ELISA)	UP664780, 200ml UP664781, 500ml UP664782, 1L		
TMB, aqueous solution (Ready-to-use for ELISA)	UPS08180, 100ml UPS08181, 500ml		See chapter "ELISA" for other ready-to-use TMB in solution.
DAB solution (50X soln + 10X dilution buffer)	UP732320, 500ml	Chromo. brown/orang e	(3,3'-DiAminoBenzimidine) widely used for IHC and WB staining. Yields a brown-orange end product, or dark brown once combined with imidazole, chromogens such as CN, or metal ions. Much more sensitive and cleaner background compared to AEC. DAB slides can be dehydrated in ethanol and cleared in xylene, then mounted for permanent record keeping. Because of its carcinogenic nature, some labs avoid the handling of DAB powder.
DAB tablets (5mg each)	UP732310 50tabs		
DAB, Ultrapure powder CAS:[868272-85-9]; MW : 360.1	UP01012G, 5g UP01012H, 10g		
TACS Blue colorimetric substrate, solution	FX8280, 3ml	Chromo. blue	A blue chromogen suitable to IHC techniques. See chapter "IHC" for more details
ADHP, Fluopure powder CAS:[119171-73-2]; MW : 257.2	FP-39423A, 5 mg FP-39423B, 25mg	Fluo. 550/585 nm $\epsilon = 54\ 000$	(10-Acetyl-3,7-Dihydroxyphenoxazine) the best fluorogenic substrate for peroxidase ! Highly specific and stable. Nearly colorless and nonfluorescent until oxidization by H_2O_2 (1:1 stoichiometry) in the presence of HRP to become a highly red fluorescent end product (resorufin). See ADHP HRP Assay Kit #HS624 in chapter A (Cell Biology).
Resazurin, Na salt, powder CAS:[62758-13-8]; MW:251.17	FP-06224B, 100mg	Fluo. 571/585 nm $\epsilon = 62\ 000$	HRP uses Resazurin (also known as Am.plex red) as an electron donor during the reduction of hydrogen peroxide to water. The resultant product, resorufin, is a highly colored and fluorescent compound. Also used as a redox indicator to probe cells (i.e. cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity in isolated neural tissue).
Luminol CAS:[521-31-3]; MW: 177.2	FP-57578A, 25g	Lum.	Gives a strong blue light flash ($\lambda_{abs}/\lambda_{em}$: 355/413nm;

Luminol, Na salt CAS:[20666-12-0]; MW: 199.1	FP-CA9611, 2.5g	355/413 nm	EC: 7650 L/mol/cm). Complex formulations are required to stabilize the glowing, and for appropriate results in ELISA or WB.
Luminol, Fluopure powder CAS: [521-31-3]; MW: 177.2	FP-04247G, 25g		
Uptilight	See our WB and ELISA dedicated pages		See other derivatives below. See also UptiLight in our ELISA and WB dedicated pages
other Peroxidase assays, kits		Fluo/lum.	See other HRP assay kits (fluorimetric, Luminometric) below in the ELISA paragraph (for ECL substrates) and in chapter A (Cell Biology) 'Apoptosis/ROS species section' for ADHP assays kit #JQ6740 for cell assays.

^(a) all products are available as bulk formats. Please inquire.

^(b) Chromo.=Chromogenic; Fluo.=Fluorigenic ; Lum.: Luminogenic

Technical tip: HRP Enzymatic substrates for immunoassays – descriptions

ABTS™ (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate) is a popular substrate for HRP, giving a soluble green end product measured at 450nm.

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 H₂O₂ (1:1 stoichiometry) in the presence of horseradish peroxidase (HRP) to become the highly red fluorescent end product (resorufin $\lambda_{ex}/\lambda_{em}(pH 9) = 571/585 \text{ nm}$; $\epsilon = 54 \text{ 000}$).

Resazurin (also known as Am.plex red) is used by Horseradish peroxidase as an electron donor during the reduction of hydrogen peroxide to water. The resultant product, resorufin, is a highly colored and fluorescent compound ($\lambda_{ex}/\lambda_{em}(pH9) : 563/587 \text{ nm}$). Also used as a redox indicator to probe cells (i.e. cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity in isolated neural tissue).

AEC (3-amino-9-ethylcarbazole) is a chromogen of peroxidase with relatively less toxic nature compared to DAB. It produces a red colored end product at the positive sites, which gives a good contrast with blue hematoxylin counter stain, and is also useful for double labeling. It is less sensitive than DAB, but exhibits less background. Slightly more sensitive than 4-CN.

4-CN (4-Chloro-1-Naphtol) yields an intense blue-black product in a easily controlled reaction. Exhibits least sensitive HRP substrate but less background than DAB.

DAB (3,3'-DiAminoBenzimidine) is a widely used chromogen for immunoperoxidase staining and immunoblotting, which yields a brown-orange end product, or dark brown once combined with imidazole, chromogens such as CN, or metal ions. It has been well accepted because of is much more sensitive and gives cleaner background as compared to AEC. Specimens stained in DAB can be dehydrated in ethanol and cleared in xylene and can be mounted for permanent record keeping. However, because of its carcinogenic nature, some labs avoid the handling of DAB powder.

Luminol as become a very useful HRP substrates in immunoassays. It gives upon reaction with H₂O₂ and HRP a strong blue light flash ($\lambda_{abs}/\lambda_{em} : 355/413 \text{ nm}$; EC: 7650 L/mol/cm). Complex formulations are required to stabilize the glowing, and for appropriate results in ELISA or Western-Blotting. Several derivatives of luminol are available, please inquire.

OPD (o-Phenylene Diamine) has been the most popular chromogenic HRP substrate until less toxic and equally sensitive chromogens were proposed (TMB), as well as even more sensitive chemiluminescent ones (Luminol, UptiLight). It is available in pre-weighed tablets for safer and more convenient use.

TACS Blue label is chromogenic substrates dedicated to IHC techniques.

TMB (3,3',5,5'-tetramethylbenzidine) has become now the most popular chromogenic substrate for detecting HRP activity, notably in ELISA. It works also in othe solution assays, blotting and immunohistochemistry. TMB produces upon reaction with peroxidase a blue color, which is measured at 370nm or 652nm, and yellow colour (stopped reaction after adding an acid - measured at 450nm). Sensitivity is greater than classic substrates like OPD and ABTS with very low background. Several derivatives exist, as TMB-z.

Other HRP substrates

TMBZ CAS[54827-17-7]; MW:240.35

TMBZ HCl CAS[64285-73-0]; MW:351.32

TMBZ-PS CAS[102062-36-2]; MW:384.48

IsoLuminol CAS:[3682-14-2]; MW: 177.2

IsoLuminol ABEI CAS: [66612-29-1]; MW: 276.34

T33080, 1g

T33140, 1g

T33120, 1g

FP-07624A, 1g

FP-60404A, 5mg

H₂O₂ Solutions

Ready-to-Use hydrogen peroxide tablets

JQ6540, 25tabs

accurately weighed, to give you an accurate hydrogen peroxide (H₂O₂, MW:34.01) concentration.

Tabs should be stored at -20°C; protected from light and moisture, and dissolve readily in water for use in HRP based Immunoassays

Ready-to-Use hydrogen peroxide solution *50 mM calibrated and stabilized soln*

JQ6550, 5x10ml

This solution is calibrated to ensure more reproducible peroxidase-based assays, using fine-calibration and a improved stabilizing formulation.

Substrates for alkaline phosphatase

Alkaline Phosphatase is preferred to Peroxidase in several applications when more sensitive and linear response or lower background is expected. Its turn-over is lower, so the staining is longer.

Alk.Phos. substrates	ref ^(a)	Type ^(b)	Comment
pNPP tablets of 30mg	UP732500, 100 tabs UP732501 1000 tabs	Chromo 405nm	(p-Nitrophenyl Phosphate) is the preferred substrate for high sensitivity detection of alkaline phosphatase in EIA assays. pNPP is a colorless and nonfluorescent substrate but produces a yellow product that absorbs at 405nm (kinetic reading, or after acid stop).
pNPP tablets of 5mg	UP89562G 100 tabs UP89562F 1000 tabs		
pNPP, Ultrapure powder CAS:[4264-83-9]; MW : 301.3	UP89562C 25 g UP89562D 100 g		
pNPP solution (ready-to-use for ELISA)	UP664791, 500ml		
Naphtol AS-MX phosphate, powder CAS:[1596-56-1]; MW: 371.33	241155, 1 g 241156, 5 g	Chromo . blue, red	an histochemical substrate commonly used in conjunction with Fast Red TR (intense red) for immunohistology, immunoblotting and dot blot applications, for the detection of alkaline phosphatase activity. Other colors (green, blue) can be obtained combining other diazonium salts.
Naphtol AS-MX phosphate Na salt	N12710, 1g N12710, 5g		
BCIP, toluidine, powder	UP10904E, 100 mg UP10904F, 500 mg	Chromo . 615nm	(5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium) is the preferred chromogenic substrate for the detection of alkaline phosphatase in immunoblotting and immunohistological staining. It yields an insoluble dark blue/purple dye precipitate ($\lambda_{max} \sim 615$ nm) and gives a variety of other colors being often combined to tetrazolium salts to amplify the sensitivity of staining.
BCIP, Na salt, powder	C47183, 100 mg	Chromo . 615nm	
BCIP Red, powder	M1636A, 100 mg	Chromo . 565nm	
BCIP Pink, powder	AM337A, 100 mg	Chromo . 540nm	
BCIP/NBT ready to use solution	UP099851, 100ml	Chrom. purple	BCIP/NBT(Nitroblue tetrazolium) solution gives an insoluble purple precipitate which color persists with minimal fading when protected from exposure to light. Ideal for in situ hybridization and assays where it contrasts with the blue hematoxylin-stained sections
BCIP Pink/NBT Kit	FI9420, 1 kit	Chrom.	contains the BCIP Pink (AM337A) and the precipitation inducer NBT (FI9430)
INT powder	UP020611, 1g	Chromo .	Used with BCIP. BCIP/INT(p-iodonitrotetrazolium) solution gives a brick red insoluble end product.
NBT powder	UP143456, 500 mg UP143457, 1g	Chromo .	Used combined with BCIP
AP-Blue, kit	CB0131, 1kit (400ml)	Chromo 490- 650nm	dark blue-puple reaction product.
AP-Red, kit	CB0141, 1kit (400ml)	Chromo 450- 550nm	orange-red reaction product.
MUP Na salt, Ultra Pure grade powder Max. fluorescence at pH >10 CAS:[22919-26-2]; MW : 277.1	FP-30045A, 100mg FP-30045B, 500mg FP-30045C, 5 g FP-30045D, 10g	Fluo. 360/440 nm	(4-Methylumbelliferyl Phosphate) forms a soluble fluorescent end product. Widely used to detect phosphatases in solution (widespread in fluorescence-detected ELISAs), but not well suited for living cell or continuous assays and acid phosphatases. MUP Plus can be used as an alternative. An hybridization assay for PCR products achieved a detection limit of 0.15 femtomoles.
MUP Free acid, powder CAS:[3368-04-5]; MW : 256.1	FP-24119A, 100 mg	Fluo. 360/440 nm	
MUP Plus, Na salt, powder Maximum fluorescence at pH > 7.0	FP-JQ6710, 25 mg	Fluo. 360/450 nm	
FDP, tetra-ammonium salt, powder CAS:[217305-49-2]; MW : 560.4	FP-72573A, 5mg	Fluo. 490/514 nm $\epsilon \approx$ 90,000	(Fluorescein DiPhosphate) is the most sensitive fluorogenic AP substrate, with green fluorescence. But it is not very thermally stable, it shows a sequential AP mediated hydrolysis, and required special cautions for storing. Also an excellent substrate for protein tyrosine phosphatase (PTPase), FDP is used in the high throughput screening (HTS) for PTPase inhibitor.. See

			also FDP ELISA kit #HT0790
other Phosphatases assays kits		Fluo/lu m.	See other AP assay kits (fluorimetric, Luminometric) in our enzyme detection/Alkaline Phosphatase section: FDP assay kit for ELISA (#JQ6740) and Cell Assays (HT0790)

^(a) all products are available as bulk formats. Please inquire.

^(b) Chromo.=Chromogenic; Fluo.=Fluorogenic ; Lum.: Luminogenic

Find other Alk.Phos. substrates in ELISA, Blotting and IHC sections. See also other phosphatases substrates such as **DiFMUP** #FP-58657A and **DDAO** #FP-73967A (do not suit well Alkaline Phosphatases), and AP enhancer #WU1960 in section D7 'IHC'.

Technical tip: AP Enzymatic substrates for immunoassays – descriptions

pNPP (p-Nitrophenyl Phosphate) is the preferred substrate for high sensitivity detection of alkaline phosphatase in EIA assays. FDP is a colorless and nonfluorescent substrate but produces a yellow product that absorbs at 405nm (kinetic reading, or after acid stop).

BCIP (5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium) is the preferred chromogenic substrate for the detection of alkaline phosphatase in immunoblotting and immunohistological staining. It yields an insoluble dark blue/purple dye precipitate ($\lambda_{\max} \sim 615$ nm) and gives a variety of other colors being often combined to tetrazolium salts to amplify the sensitivity of staining. The **BCIP/NBT**(Nitroblue tetrazolium) solution gives an insoluble purple precipitate onto the site where the enzyme-labeled antibody which color persists with minimal fading when protected from exposure to light. The **BCIP/INT**(p-iodonitrotetrazolium) solution gives a brick red color. Derivates of BCIP exist to yield colored red precipitate ($\lambda_{\max} \sim 565$ nm), pink precipitate ($\lambda_{\max} \sim 540$ nm), violet.... See the sections 'ELISA', 'Blotting' and 'IHC'. **BCIP/FastRed TR** yields a red color more appropriate for dual color i.e. in microarray.

Naphthol AS-MX phosphate is an histochemical substrate commonly used in conjunction with Fast Red TR (intense red) for immunohistology, immunoblotting and dot blot applications, for the detection of alkaline phosphatase activity. Other colors (green, blue) can be obtained combining other diazonium salts.

FDP (fluorescein diphosphate) is the most sensitive fluorogenic alkaline phosphatase substrate, with green fluorescence [end product $\lambda_{\text{ex}} \lambda_{\text{em}} = 490/514$ nm; $\epsilon \approx 90,000$]. FDP is a colorless and nonfluorescent substrate for alkaline phosphatases. However FDP is not very thermally stable, shows a sequential alkaline phosphatase mediated hydrolysis, and required special cautions for storing. Also an excellent substrate for protein tyrosine phosphatase (PTPase), FDP is used in the high throughput screening (HTS) for PTPase inhibitor.

MUP (4-Methylumbelliferyl Phosphate) is a substrate for alkaline phosphatase that forms a soluble fluorescent end product. Measure MUP fluorescence by excitation at 360 nm and emission at 440 nm. It is widely used for detecting phosphatases in solution (widespread in fluorescence-detected ELISAs), but not well suited for living cell or continuous assays and acid phosphatases. As alternative, the MUP Plus can be used. An hybridization assay for PCR products achieved a detection limit of 0.15 femtomoles.

Substrates for osidases (β -galactosidase, β -glucuronidase, β -glucosidase)

Substrates for β -galactosidase and other osidases (β -glucuronidase, β -glucosidase) are mainly used in reporter assays, for colony identification, and in IHC, rather than for immuno-based detections. They are presented in our 'Genomics' chapter .

Substrates for Acetyl choline esterase (AChE) information

Acetylcholinesterase (AChE) is an enzyme that hydrolyse substrates to produce choline and an acetate group . AChE is used in immunoassays because of its extremely quick turnover ^(note) , beside other applications.

Please [check here AChE products](#) , and [ask](#) if you need AChE labeled antibodies.

See also [ELISA kits page](#): Cayman kit include AChE labeled antibodies)

Related products

Immunoreagents > [Blotting](#) (membranes) {BLW}| [ELISA](#) (μ plates) {ELI}| [MicroArray](#) (slides) {MAR}| [Buffers](#) {BBM}| [Blocking agents](#) {ILA/sat}| [Biotinylation reagents and kits](#) {LAK}|

Extended list of enzymatic substrates

{table ENS} (x)

Article	Brand	Reference	Product	Qty
JQ6581	AAT BIOQUEST	JQ6581	NEAR INFRARED FLUOROGENIC PEROXIDASE SUBSTRATE (647/670NM)	1 x 1 E
236643	ALPHA DIAGNOSTIC	SP-101392-1	SUPERIOR ALTERNATIVE TO ADHP (AMPLEX RED)	1 x 1 E
JW8791	INT	SP-100796-1	PHOSPHATASE SUBSTRATE	1 x 1 E
JW8791	ALPHA DIAGNOSTIC	SP-100796-1	2A/2B DENGUE PROTEASE SUBSTRATE	1 x 1 E
JW8802	INT	SP-100800-1	3/4A, DENGUE PROTEASE SUBSTRATE	1 x 1 E

JX1071	ALPHA DIAGNOSTIC INT	SP-100797-1	2B/3, DENGUE PROTEASE SUBSTRATE	1 x 1 E
JX1402	ALPHA DIAGNOSTIC INT	SP-53571-1	ABL CYTOSOLIC SUBSTRATE	1 x 1 E
ZS2982	ALPHA DIAGNOSTIC INT	SP-102104-5	P60C-SRC SUBSTRATE II	1 x 5 E
ZS3822	ALPHA DIAGNOSTIC INT	SP-101102-1	MMP-2/MMP-9 SUBSTRATE I, FLUOROGENIC	1 x 1 E
ZS3832	ALPHA DIAGNOSTIC INT	SP-101104-1	MMP-3 SUBSTRATE I, FLUOROGENIC	1 x 1 E
RA3865	ALPHA DIAGNOSTIC INT	SP-52262-1	HIV PROTEASE SUBSTRATE	1 x 1 E
AJN450	ALPHA DIAGNOSTIC INT	SP-101339-2	CASPASE 1 SUBSTRATE 1M (ICE), FLUOROGENIC, AC-YEVD-AMC	1 x 2 E
AJN460	ALPHA DIAGNOSTIC INT	SP-101341-2	CASPASE 1 SUBSTRATE 2M (ICE), FLUOROGENIC, AC-YVAD-AMC	1 x 2 E
AJN470	ALPHA DIAGNOSTIC INT	SP-101342-2	CASPASE 2 SUBSTRATE 1M (ICH-1), FLUOROGENIC, AC-VDVAD-AMC	1 x 2 E
AJN480	ALPHA DIAGNOSTIC INT	SP-101344-2	CASPASE 3 SUBSTRATE 1M (APOPAIN), FLUOROGENIC, AC-DEVD-AMC	1 x 2 E
AJN490	ALPHA DIAGNOSTIC INT	SP-100802-2	CATHEPSIN S SUBSTRATE	1 x 2 E
AJN750	ALPHA DIAGNOSTIC INT	SP-101395-1	PHOSPHORYLATED PROTEIN KINASE C SUBSTRATE 2	1 x 1 E
AJT160	ALPHA DIAGNOSTIC INT	RP-982	HUMAN RAS-RELATED C3 BOTULINUM TOXIN SUBSTRATE 1	1 x 10 B
AJU310	ALPHA DIAGNOSTIC INT	SP-64991-5	MBP MAPK SUBSTRATE	1 x 5 E
AJU850	ALPHA DIAGNOSTIC INT	SP-101394-1	PHOSPHORYLATED PROTEIN KINASE C SUBSTRATE 1	1 x 1 E
AJV330	ALPHA DIAGNOSTIC INT	SP-56189-5	TRANSGLUTAMINASE SUBSTRATE, BIOTINYLATED	1 x 5 E
ZS2972	ALPHA DIAGNOSTIC INT	SP-102105-5	P60C-SRC SUBSTRATE II, PHOSPHORYLATED	1 x 5 E
AJY190	ALPHA DIAGNOSTIC INT	SP-86880-5	AKT/PKB/RAC - PROTEIN KINASE SUBSTRATE	1 x 5 E
AJZ830	ALPHA DIAGNOSTIC INT	SP-101517-5	BIOTIN-LC-PROTEIN KINASE G SUBSTRATE, BIOTIN-LC-G-SUBTIDE	1 x 5 E
AJZ920	ALPHA DIAGNOSTIC INT	SP-101518-5	BIOTIN-RR-SRC, INSULIN RECEPTOR TYROSINE KINASE SUBSTRATE	1 x 5 E
AKA110	ALPHA DIAGNOSTIC INT	SP-101338-5	CASPASE 1 SUBSTRATE 1 (ICE), CHROMOGENIC, AC-YEVD-PNA	1 x 5 E
AKA120	ALPHA DIAGNOSTIC INT	SP-101340-5	CASPASE 2 SUBSTRATE, CHROMOGENIC, AC-VDQD-PNA	1 x 5 E
AKA130	ALPHA DIAGNOSTIC INT	SP-101343-5	CASPASE 3 SUBSTRATE, CHROMOGENIC, AC-VQVD-PNA	1 x 5 E
AKD120	ALPHA DIAGNOSTIC INT	SP-89575-5	HUMAN CMV ASSEMBLIN PROTEASE SUBSTRATE (M-SITE)	1 x 5 E
AKF050	ALPHA DIAGNOSTIC INT	SP-101823-5	INTERLEUKIN-1BETA CONVERTASE SUBSTRATE	1 x 5 E
AKF600	ALPHA DIAGNOSTIC INT	SP-101100-5	MMP BIOTINYLATED SUBSTRATE I	1 x 5 E
AKG490	ALPHA DIAGNOSTIC INT	SP-101516-5	PP60(V-SRC) AUTOPHOSPHORYLATION SITE, PROTEIN TYROSINE KINASE SUBSTRATE	1 x 5 E
AKG660	ALPHA DIAGNOSTIC INT	SP-101520-5	PROTEIN KINASE C SUBSTRATE, GLYCOGEN SYNTHASE (1-8)	1 x 5 E
AKG670	ALPHA DIAGNOSTIC INT	SP-59761-5	PROTEIN KINASE G SUBSTRATE, G-SUBTIDE	1 x 5 E
AKH110	ALPHA DIAGNOSTIC INT	SP-101553-5	RSK SUBSTRATE, S6 (231-239)	1 x 5 E
S29595	ALPHA DIAGNOSTIC INT	80100	STOP SOLUTION FOR TMB IN ELISA 450NM READING	1 x 50 X
BV3021	AMRESCO	BV3021	VisiGLO AP Substrate (for 2000cm2)	1 x 100 X
O2673F	AMRESCO	O2673F	O-PHENYLENEDIAMINE	1 x 25 G
O36310	AMRESCO	O36310	3-AMINO-9-ETHYLCARBAZOLE	1 x 50 G
O2673G	AMRESCO	O2673G	O-PHENYLENEDIAMINE	1 x 50 G

270861	AMRESKO	270861	OPD Tablets, Biotechnology grade	1 x 50 k
02061D	AMRESKO	02061D	2-(P-IODOPHENYL)-3-(P-NITROPHENYL)-5-PHENYLTETRAZO	1 x 500 E
895622	AMRESKO	895622	4-NITROPHENYL PHOSPHATE DISODIUM SALT, 5mg pNPP TABLETS	1 x 100 k
732502	AMRESKO	732502	4-NITROPHENYL PHOSPHATE DISODIUM SALT TABLETS OF 30mg of pNPP)	1 x 100 k
895623	AMRESKO	895623	4-NITROPHENYL PHOSPHATE DISODIUM SALT, 5mg pNPP TABLETS	1 x 1 Q
732503	AMRESKO	732503	4-NITROPHENYL PHOSPHATE DISODIUM SALT TABLETS OF 30mg of pNPP)	1 x 1 Q
BP7080	ANASPEC	BP7080	ALKALINE PHOSPHASE ELISA ASSAY PNPP COLORIMETRIC (405NM) WITH AP-GOAT ANTI-MOUSE IGG	1 x 1 A
236642	ANASPEC	236642	PHOSPHATASE SUBSTRATE	1 x 1 E
236640	ANASPEC	236640	PHOSPHATASE SUBSTRATE	1 x 5 E
JQ498X	ANASPEC	23887	EDANS ACID [5-((2-AMINOETHYL)AMINO)NAPHTHALENE-1-SULFONIC ACID]	1 x 1 G
IS9570	ANASPEC	IS9570	HILYTE FLUOR 488-B-AMYLOID (1-28)	1 x .1 E
JW9190	ANASPEC	JW9190	CASPASE 1 (ICE) SUBSTRATE FOR FRET ASSAYS	1 x 1 E
IS9231	ANASPEC	IS9231	BIOTIN-DADEY(PO3)LIPQQG	1 x 5 E
S51681	BIOFX	S51681	STOP SOLUTION FOR TMB IN ELISA 650nm reading	1 x 100 X
S51682	BIOFX	S51682	STOP SOLUTION FOR TMB IN ELISA 650nm reading	1 x 1 L
CV7530	BIOFX	CV7530	CHEMILUMINESCENT AP SUBSTRATE UltraSentivive, 450nm reading	1 x 100 X
CV7531	BIOFX	CV7531	CHEMILUMINESCENT AP SUBSTRATE UltraSentivive, 450nm reading	1 x 250 X
CV7532	BIOFX	CV7532	CHEMILUMINESCENT AP SUBSTRATE UltraSentivive, 450nm reading	1 x 500 X
830635	IMMUNOBIOSCIENCE	AR-8201-01	AEC SINGLE SOLUTION	1 x 200 X
FM2071	IMMUNOBIOSCIENCE	AR-8202-01	DAB (2 COMPONENTS)	1 x 20 k
02673A	PIERCE	34005	O-PHENYLENEDIAMINE	1 x 25 G
00749A	PIERCE	34010	4-CHLORO-1-NAPHTHOL	1 x 25 G
007499	PIERCE	34011	4-CHLORO-1-NAPHTHOL	50 x 30 E
143453	PIERCE	34035	NITRO BLUE TETRAZOLIUM CHLORIDE : NBT	1 x 1 G
270864	PIERCE	34006	O-PHENYLENEDIAMINE OPD, TABLETS	50 x 5 E
Q89572	PIERCE	N301	TMB SUBSTRATE SOLUTION	1 x 100 X
XT2150	SPRING BIOSCIENCE	DAB-060	DAB (2 COMPONENTS)	1 x 60 X
FM2070	SPRING BIOSCIENCE	DAB-125	DAB (2 COMPONENTS)	1 x 125 X
FM2110	SPRING BIOSCIENCE	DFN-125	FAST RED (2 COMPONENT)	1 x 125 X
MJ2360	SPRING BIOSCIENCE	LFR-125	LIQUID FAST RED SUBSTRATE SYSTEM (75X)	1 x 125 X
830631	SPRING BIOSCIENCE	ASS-125	AEC SINGLE SOLUTION	1 x 125 X
MJ2361	SPRING BIOSCIENCE	LFR-999	LIQUID FAST RED SUBSTRATE SYSTEM (75X)	1 x 1 L
YR0820	SPRING BIOSCIENCE	LFRD-999	LIQUID FAST RED SUBSTRATE SYSTEM (25X)	1 x 1 L
FX8280	TREVIGEN	FX8280	TACS BLUE LABEL	1 x 3 X
Q69650	TREVIGEN	Q69650	TACS BLUE LABEL DETECTION MODULE	1 x 30 t
C47120	BIOSYNTH AG	C47120	4-METHYLUMBELLIFERYL PHOSPHATE DI-(CYCLOHEXYLAMMON	1 x 500 E
BT6954	BIOSYNTH AG	BT6954	4-METHYLUMBELLIFERYL PHOSPHATE DILITHIUM SALT	1 x 500 E
C47121	BIOSYNTH AG	C47121	4-METHYLUMBELLIFERYL PHOSPHATE DI-(CYCLOHEXYLAMMON	1 x 1 G
C47122	BIOSYNTH AG	C47122	4-METHYLUMBELLIFERYL PHOSPHATE DI-(CYCLOHEXYLAMMON	1 x 2.5 G
C47123	BIOSYNTH AG	C47123	4-METHYLUMBELLIFERYL PHOSPHATE DI-(CYCLOHEXYLAMMON	1 x 5 G
C47112	BIOSYNTH AG	C47112	4-METHYLUMBELLIFERYL PHOSPHATE DI-(2-AMINO-2-METHYL-1,3-PROPANEDIOL)SALT	1 x 100 E
C47073	BIOSYNTH AG	C47073	PHOSPHOENOL PYRUVATE TRISODIUM SALT	1 x 500 E
C47111	BIOSYNTH AG	C47111	4-METHYLUMBELLIFERYL PHOSPHATE DI-(2-AMINO-2-METHYL-1,3-PROPANEDIOL)SALT	1 x 250 E
BT6955	BIOSYNTH AG	BT6955	4-METHYLUMBELLIFERYL PHOSPHATE DILITHIUM SALT	1 x 1 G
C47113	BIOSYNTH AG	C47113	4-METHYLUMBELLIFERYL PHOSPHATE DI-(2-AMINO-2-METHYL-1,3-PROPANEDIOL)SALT	1 x 500 E
C47074	BIOSYNTH AG	C47074	PHOSPHOENOL PYRUVATE TRISODIUM SALT	1 x 2.5 G
BT6956	BIOSYNTH AG	BT6956	4-METHYLUMBELLIFERYL PHOSPHATE DILITHIUM SALT	1 x 2.5 G
BT6957	BIOSYNTH AG	BT6957	4-METHYLUMBELLIFERYL PHOSPHATE DILITHIUM SALT	1 x 5 G

154262	BIOSYNTH AG	154262	3,3',5,5'-TETRAMETHYLBENZIDINE DIHYDROCHLORIDE (TMB)	1 x 1 G
007492	BIOSYNTH AG	007492	4-CHLORO-1-NAPHTHOL	1 x 100 E
007493	BIOSYNTH AG	007493	4-CHLORO-1-NAPHTHOL	1 x 250 G
MM6610	CAYMAN CHEMICAL	MM6610	RENIN FLUOROGENIC SUBSTRATE	1 x 1 E
VY1700	CAYMAN CHEMICAL	10010208	ACTIVE CASPASE-3 SUBSTRATE N-AC-DEVD-N'-MC-R110	1 x 1 U
QZ8953	GENSCRIPT CORP.	L00222V60	CHROMOSENSOR ONE-SOLUTION TMB SUBSTRATE	1 x 60 X
RA6510	GENSCRIPT CORP.	RP20113	PKG, PHOSPHO (SER4) SUBSTRATE	1 x .5 E
QZ8950	GENSCRIPT CORP.	L00222V125	CHROMOSENSOR ONE-SOLUTION TMB SUBSTRATE	1 x 125 X
JX1401	GENSCRIPT CORP.	RP19985	ABL CYTOSOLIC SUBSTRATE	1 x .5 E
RA6460	GENSCRIPT CORP.	RP20107	CDK5, PHOSPHO (THR3) SUBSTRATE	1 x .5 E
RA3860	GENSCRIPT CORP.	RP17318	HIV PROTEASE SUBSTRATE	1 x 1 E
RA6480	GENSCRIPT CORP.	RP20109	EGFR2, PHOSPHO (TYR5) SUBSTRATE	1 x .5 E
67992i	LEINCO TECHNOLOGIES	K107	METAL ENHANCED DAB SUBSTRATE KIT	1 x 300 X
HT079A	MARKER GENE TECHNO.	HT079A	FLUORESCENT ALKALINE PHOSPHATASE ASSAY KIT (FDP SUBSTRATE)	1 x 100 t
VD6600	SEROTEC	VD6600	AEC SUBSTRATE BUFFER	2 x 50 X
VD6620	SEROTEC	VD6620	DAB SUBSTRATE BUFFER	2 x 50 X
VD6640	SEROTEC	VD6640	FAST RED SUBSTRATE BUFFER	2 x 50 X
VD6630	SEROTEC	VD6630	FAST RED SUBSTRATE TABLETS	1 x 20 U
C47079	SIGMA	C47079	PHOSPHOENOL PYRUVATE TRISODIUM SALT	1 x 500 B
IY7641	APPLIED BIOSYSTEMS	T2305	CHEMILUMINESCENT AP SUBSTRATE CDP-STAR CONCENTRATE 12.5MM	1 x 2 X
IY6511	APPLIED BIOSYSTEMS	T2041	CHEMILUMINESCENT AP SUBSTRATE CSPD STAR, CONCENTRATE 25MM	1 x 2.5 X
IY7642	APPLIED BIOSYSTEMS	T2306	CHEMILUMINESCENT AP SUBSTRATE CDP-STAR CONCENTRATE 12.5MM	1 x 5 X
IY7643	APPLIED BIOSYSTEMS	T2307	CHEMILUMINESCENT AP SUBSTRATE CDP-STAR CONCENTRATE 12.5MM	1 x 10 X
IY6513	APPLIED BIOSYSTEMS	T2043	CHEMILUMINESCENT AP SUBSTRATE CSPD STAR, CONCENTRATE 25MM	1 x 10 X
IY7644	APPLIED BIOSYSTEMS	T2308	CHEMILUMINESCENT AP SUBSTRATE CDP-STAR CONCENTRATE 12.5MM	1 x 20 X
IY6514	APPLIED BIOSYSTEMS	T2044	CHEMILUMINESCENT AP SUBSTRATE CSPD STAR, CONCENTRATE 25MM	1 x 25 X
IY7645	APPLIED BIOSYSTEMS	T2309	CHEMILUMINESCENT AP SUBSTRATE CDP-STAR CONCENTRATE 12.5MM	1 x 50 X
RK4371	BIOFX	SERI-0110-2C	CHEMILUMINESCENT SENSITIVE HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4372	BIOFX	SERI-0220-2C	CHEMILUMINESCENT SENSITIVE HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4360	BIOFX	LERI-0050-2C	CHEMILUMINESCENT SENSITIVE PLUS HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4361	BIOFX	LERI-0110-2C	CHEMILUMINESCENT SENSITIVE PLUS HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4373	BIOFX	SERI-0440-2C	CHEMILUMINESCENT SENSITIVE HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4362	BIOFX	LERI-0220-2C	CHEMILUMINESCENT SENSITIVE PLUS HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4400	BIOFX	APS4-0100-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (450 NM)	1 x 1 U
RK4410	BIOFX	APS5-0100-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (540 NM)	1 x 1 U
RK4374	BIOFX	SERI-1000-2C	CHEMILUMINESCENT SENSITIVE HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4363	BIOFX	LERI-0440-2C	CHEMILUMINESCENT SENSITIVE PLUS HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4401	BIOFX	APS4-0250-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (450 NM)	1 x 1 U
RK4411	BIOFX	APS5-0250-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (540 NM)	1 x 1 U
RK4364	BIOFX	LERI-1000-2C	CHEMILUMINESCENT SENSITIVE PLUS HRP MICROWELL AND/OR MEMBRANE SUBSTRATE	1 x 1 U
RK4402	BIOFX	APS4-0500-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (450 NM)	1 x 1 U
RK4412	BIOFX	APS5-0500-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (540 NM)	1 x 1 U
RK4380	BIOFX	APU4-0500-01	CHEMILUMINESCENT ULTRA SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (450 NM)	1 x 1 U
RK4390	BIOFX	APU5-0500-01	CHEMILUMINESCENT ULTRA SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (540 NM)	1 x 1 U
RK4403	BIOFX	APS4-1000-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (450 NM)	1 x 1 U
RK4413	BIOFX	APS5-1000-01	CHEMILUMINESCENT SUPER SENSITIVE AP MICROWELL AND/OR MEMBRANE SUBSTRATE (540 NM)	1 x 1 U

Related products/documents

Enzyme substrates (incl. [TMB solution](#), pNPP solution, OPG)

Accessory reagents for HRP and AP detections

Peroxidase Enhancer

WU1920, 250ml

Alkaline Phosphatase Enhancer

WU1960, 250 ml

Hydrogen Peroxide block

FM2155, 60ml

FM2150, 100ml

quench endogenous peroxidase that can cause nonspecific background. Peroxidase within red blood cells may be difficult to quench entirely.

Endogenous Alkaline phosphatase block

MJ2354, 15 ml

MJ2355, 50 ml

Enzyme labeled (Strept)Avidin products

Enzyme/crosslinkers and enzyme labeling kits for antibodies and other biomolecules

Other labeled secondary antibodies and primary antibodies

Buffers and saturating agents

see [Products HighLights \(overview\)](#)

see [BioSciences Innovations catalog](#)

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