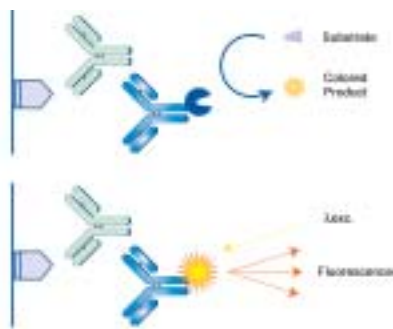


Immunologicals - Accessory reagents

Enzymatic Substrates



Enzymes represent 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 now standard in immunoassays. Substrates are converted in colored or light emitting products detected by spectrophotometry or 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.

Selection guide

Enzymatic substrates in immunodetections

Enzyme	Substrates	Applications	+	-
(HRP) Peroxidase			Rapid kinetic Numerous substrates available	Back ground in blood samples and some tissues Staining stability lower than AP
	Chromogenic, soluble (TMB, ABTS, OPD...)	ELISA/HRP	Easy to use, popular	Sometimes light sensitive coloration
	Chromogenic, precipitating (CN, AEC, DAB,...)	WB, SB, IHC/HRP	Easy to use, popular	
	Fluorogenic (ADHP/resorufin)			
	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			More sensitive and linear response	Often less economic than HRP. High molecular weight conjugates
	AP Chromogenic, soluble (pNPP)	ELISA/AP	Linear kinetic Often more sensitive than HRP	
	AP Chromogenic, precipitating (BCIP/NBT,...)	WB, SB, IHC/AP	Stability of staining	Back ground in some samples
	Fluorogenic (MUP, FPD)	FLISA, IHF	Sensitivity	Need fluorescence expensive equipment
Oxidases (b-galactosidase,...)				
	Chromogenic substrates (X-Gal, ONG,...)	GRA, IHC	High sensitivity	
	Fluorogenic (MUG)	GRA, IHF	High sensitivity	

A.356

WB : Western Blotting ; SB : Southern Blotting ; IHC : ImmunoHistoChemistry ; ELISA : Enzyme Linked Immunosorbent Assay ; GRA : Gene Reporter Assay

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).

Please refer to section "Elisa technique", "Blotting technique", "IHC technique" for optimized substrates solutions and kits

ABTS™

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate) reacts with HRP and H_2O_2 to produce a soluble green end product that can be measured at 450nm.

Description	Cat.#	Qty
ABTS, Liquid substrate	UP732550	100 ml
Ready-to-use solution	UP732551	500 ml
ABTS tablets	42387C	50 tablets
Tablet 10/140 mg each contain 10 mg of ABTS for quick and easy preparation of substrate solution.		
ABTS, Ultra pure grade	UP423876	1 g
$C_{18}H_{24}N_6O_6S_4$; MW : 548.68	UP423877	10 g

ADHP (10-Acetyl-3,7-dihydroxyphenoxazine)

ADHP 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 (1:1 stoichiometry) in the presence of horseradish peroxidase (HRP) to become the highly red fluorescent resorufin. High purity resorufin is also available to be used as a standard.

See also ADHP ELISA kit # HS6240 page A366

$\lambda_{ex}/\lambda_{em}$ (pH 9) : 571/585 nm (end product)

ϵ : 54 000 (end product)

White to off-white solid, soluble in DMSO or DMF

Description	Cat.#	Qty
ADHP	FP-39423A	5 mg
$C_{14}H_{11}NO_4$; MW : 257.24		

Resorufin

High purity resorufin that can be used as a standard for enzyme substrates whose end product is resorufin.

Dark solid, soluble in DMSO

$\lambda_{ex}/\lambda_{em}$ (pH9) : 563/587 nm

Description	Cat.#	Qty
Resorufin	FP-95432B	100 mg
$C_{12}H_7NO_3$; MW : 213.04		

AEC

3-amino-9-ethylcarbazole (AEC) is a chromogen of choice when performing immunoperoxidase. It's 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.

Less sensitive than DAB, but exhibits less background. Slightly more sensitive than 4-CN. The red deposit formed makes AEC useful for double labeling.

AEC formulated substrates

See the description in section "IHC" page A377

Description	Cat.#	Qty
AEC, UltraPure powder	036310	50 g
$C_{14}H_{14}N_2$; MW : 310.28		

4-CN

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

Description	Cat.#	Qty
4-CN, UltraPure powder	UP007490	25 g
$C_{10}H_7ClO$; MW : 178.62	UP007491	50 g

Immunologicals - Accessory reagents

Enzymatic Substrates

DAB

3,3'-DiAminoBenzimidine (DAB) is a widely used chromogen for immunoperoxidase staining and immunoblotting. It has been well accepted because of its superior performance as compared to amino ethylcarbazole (AEC). DAB is much more sensitive and gives cleaner background as opposed 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.

Yields a brown-orange end product.

Description	Cat.#	Qty
DAB liquid	UP732320	500 ml
Ready-to-use solution less toxic for manipulation Supplied as a 50x concentrate with a 10x dilution buffer.		
DAB tablets	UP732310	50 tablets
Contains 5mg/tablet	UP732311	100 tablets
DAB Ultra Pure powder	UP01012G	5 g
$C_{12}H_{14}N_4 \cdot HCl$; MW : 360.12	UP01012H	10 g

Luminol

Luminol as become a very useful HRP substrates in immunoassays. Complex formulations are required for appropriate results in ELISA or Western-Blotting.

See the description of UptiLight™ WB chemiluminescent substrate in section "Blotting" page A374.

OPD

o-Phenylene Diamine (OPD) has been the most popular HRP substrate until less toxic and equally sensitive substrates were proposed (TMB, chemiluminescence). OPD is available in pre-weighed 15 mg tablets for safer and more convenient use.

Description	Cat.#	Qty
OPD powder	02673F	25 g
$C_6H_8N_2$; MW : 108.14	02673G	50 g
OPD tablets 15 mg	270861	50 tabs

TACS Blue label

TACS Blue label is chromogenic substrates dedicated to IHC techniques.

Description	Cat.#	Qty
TACS Blue colorimetric substrate	FX8280	3 ml
TACS Blue label detection module	Q69650	30 tests
Includes TACS Blue substrate (3 ml) and Nuclear Fast Red counter stain.		

TMB formulated substrates
See the description of our optimized TMB solutions in section "ELISA" page A365.

TMB (TetraMethylBenzidine)

TMB is a popular substrate for detecting HRP activity. It is useful for ELISA and solution assays, as well as for blotting.

Produces upon reaction with peroxidase a blue color, which is measured at 370 nm or 652 nm, and yellow colour (stopped reaction after adding an acid (measured at 450 nm))

Sensitivity is greater than classic substrates like OPD and ABTS with very low background.

Description	Cat.#	Qty
TMB powder, UltraPure	UP15426D	1 g
$C_{16}H_{24}N_2OCl_2$; MW : 331.29	UP15426E	5 g
Suits for ELISA and blotting		

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.

BCIP, INT, NBT

5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP) is the preferred chromogenic substrate for the detection of alkaline phosphatase in immunoblotting and immunohistological staining. It is often combined to p-iodonitrotetrazolium (INT) or Nitroblue tetrazolium (NBT) to amplify the sensitivity of staining.

Description	Cat.#	Qty
BCIP, toluidine	UP10904E	100 mg
	UP10904F	500 mg
BCIP, Na dark blue (λ_{max} ~615 nm)	C47183	100 mg
BCIP Red derivative of BCIP, a red (λ_{max} ~ 565 nm) colored precipitate	M1636A	100 mg
BCIP Pink derivative of BCIP, pink colored (λ_{max} ~ 540 nm) precipitate	AM337A	100 mg

INT

Description	Cat.#	Qty
INT	UP020611	1 g

NBT

Description	Cat.#	Qty
NBT	UP143456	500 mg
	UP143457	1 g

BCIP/NBT ready to use solution

The most commonly used alkaline phosphatase substrate is 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT). When the dephosphorylation of BCIP is catalyzed by alkaline phosphatase, an insoluble purple dye is precipitated onto the site where the enzyme-labeled antibody is bound to target antigen. The color persists with minimal fading when protected from exposure to light.

NBT/BCIP formulated substrates
See other kits in section "ELISA", "Blotting" and "IHC".

Description	Cat.#	Qty
BCIP/NBT	UP099851	100 ml
BCIP Pink/NBT Kit Each kit contains the BCIP Pink (AM337A) and the precipitation inducer NBT (F19430)	F19420	1 kit
BCIP/INT ready to use solution	UP096051	100 ml

The brick red insoluble end product is ideal for in situ hybridization and assays where it contrasts with the blue hematoxylin-stained sections.

MUP

4-Methylumbelliferyl Phosphate (MUP) 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.

$\lambda_{ex}/\lambda_{em}$: 360/440 nm

Description	Cat.#	Qty
MUP Na	30045A	100 mg
	30045C	5 g
	30045D	10 g
MUP Free acid	FP-24119A	100 mg

Immunologicals - Accessory reagents

Enzymatic Substrates

See also FDP complete ELISA kit # HT0790 page A366.

FDP (fluorescein diphosphate, tetraammonium salt)

FDP is the most sensitive fluorogenic alkaline phosphatase substrate. Also an excellent substrate for protein tyrosine phosphatase (PTPase), FDP is used in the high throughput screening (HTS) for PTPase inhibitor.

$\lambda_{ex} \lambda_{em}$: 490/514 nm (end product)

$\epsilon \sim 90\,000$ (end product)

Description	Cat.#	Qty
FDP	FP-72573A	5 mg

Naphtol

Naphtol AS-MX phosphate is a histochemical substrate used for the detection of alkaline phosphatase activity. Commonly used in conjunction with fast red TR for immunohistology, immunoblotting and dot blotting applications.

Description	Cat.#	Qty
Naphtol AS-MX phosphate, High Purity powder	N12710	1 g
	N12711	5 g
1-Naphtyl phosphate, Na salt, Monohydrate, High Purity	N12710	1 g
	N12711	5 g

pNPP (p-Nitrophenyl Phosphate)

Is the preferred substrate for high sensitive detection of alkaline phosphatase in EIA assays. It produces a yellow product that absorbs at 405 nm.

See also pNPP ELISA complete kit # BP7080 page A365.

Description	Cat.#	Qty
pNPP 5/175mg tablets	UP89562G	100 tabs
	UP89562F	1000 tabs
each tablet contains 5 mg of pNPP for quick and easy preparation of substrate solution.		
pNPP 30/175mg tablets	UP732500	100 tabs,
	UP732501	1000 tabs
each tablet contains 30 mg of pNPP for quick and easy preparation of substrate solution.		
PNPP powder	UP89562C	25 g
	UP89562D	100 g

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

Substrates for glucosidase are mainly used in reporter assays, for colony identification, and in IHC, more rarely for immuno-based detections. They are presented in sectionD "Genomic".

Description	Cat.#	Qty
o-NPG (o-Nitrophenyl- β -D-galactopyranoside (chromogenic)	UP556683	5 g