LIFE SCIENCE

Substrates for Biochemical Assays

For Alkaline Phosphatase

4-Nitrophenyl Phosphate (Ready-to-use solution) [for ELISA]
(= **pNPP** (Ready-to-use solution)) 100mL [N1109]

Application

1. Add 100µL of pNPP solution (Product No. **N1109**) to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. To terminate the reaction, add 100 µL of 1N NaOH solution (Product No. **S0542**) to each well.
4. Within 1 hour from start the reaction, measure the absorbance of each well at 405 nm.

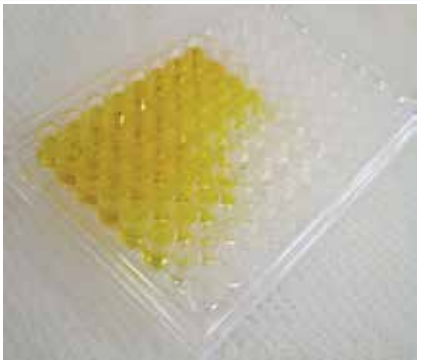

Figure. An example of use by the above method

Soluble Substrates

4-Nitrophenyl Phosphate Disodium Salt Hexahydrate (= pNPP)	1g / 5g	[D4005]
4-Nitrophenyl Phosphate Di(tris) Salt	5g	[N0422]
1-Naphthylphosphoric Acid Monosodium Salt Monohydrate	1g / 5g / 25g	[N0452]
1-Naphthylphosphoric Acid Disodium Salt Hydrate	1g / 5g	[P0263]

Precipitate Substrates

For such as immunohistochemical staining or immunoblotting, substrates arising precipitate dyes with alkaline phosphatase.

Fast Blue RR Salt	5g / 25g	[B0785]
X-Phosphate *p*-Toluidine Salt	100mg / 1g	[B1239]
Blue Tetrazolium	1g / 5g	[B3581]
Naphthol AS-TR Phosphate	200mg	[C2250]
Nitro Blue Tetrazolium (= NBT)	100mg / 1g	[D0844]
Iodonitrotetrazolium Chloride (= INT)	100mg / 1g	[I0781]
Tetranitro Blue Tetrazolium (= TNBT)	100mg / 1g	[T0250]

For Peroxidase

TMB [for ELISA] (Ready-to-use solution)

(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))

100mL [T3854]

Application

1. Add 100µL of TMB solution (Product No. T3854) to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. The reaction, add 100µL of 1N HCl solution (Product No. H1202) to each well.
4. Measure the absorbance of each well at 450 nm.

When this product T3854 reacts with horseradish peroxidase (HRP), a blue colored soluble reaction product appears thus it can be used for ELISA.
This product cannot be used for Western blotting which needs a precipitate.

Figure.
An example of use by the above method

TMB [for Western blotting] (Ready-to-use solution)

(= 3,3',5,5'-Tetramethylbenzidine (Ready-to-use solution))

100mL [T3855]

Application

1. Incubate a blotting membrane with an HRP-conjugated antibody and then wash the membrane.
2. Incubate the washed membrane with TMB solution (Product No. T3855) until color development.
3. Add deionized water to stop color development.

When this product T3855 reacts with HRP, a blue-purple precipitate appears thus it can be used for Western blotting.
This product cannot be used for ELISA which needs a soluble reaction product.

M 1

Figure.
An example of Western blotting by the above method

M : molecular weight marker
1 : Target protein A

4-CN (Ready-to-use solution) [for Western blotting]

(= 4-Chloro-1-naphthol (Ready-to-use solution))

100mL [C3384]

Application

1. Incubate a blotting membrane with an HRP-conjugated antibody and then wash the membrane.
2. Incubate the washed membrane with 4-CN solution (Product No. **C3384**) until color development.
3. Add deionized water to stop color development.

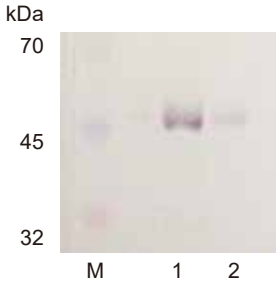

Figure.
An example of Western blotting by the above method

M : molecular weight marker
1 : Target protein B (Middle concentration)
2 : Target protein B (Low concentration)

AzBTS (Ready-to-use solution) [for ELISA]

(= 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt) (Ready-to-use solution))

100mL [A3176]

Application

1. Add 100µL of AzBTS solution (Product No. **A3176**) to each well.
2. Incubate the plate at room temperature for 30 minutes.
3. Within 1 hour from start the reaction, measure the absorbance of each well at 405 nm.

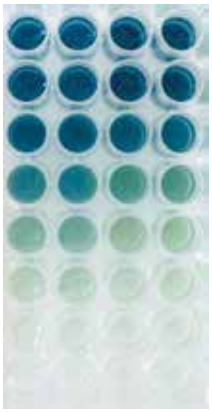

Figure.
An example of use by the above method

DAB staining kit

1kit [D5909]

Advantage

DAB substrate solution is used for detecting HRP-conjugated antibodies by Western blot analysis. DAB staining kit includes three kinds of reagents which are necessary for preparing the DAB substrate solution. The substrate solution is easily prepared by mixing each component. This kit includes reagents for 100 tests (about 500 mL) of the DAB substrate solution.

Application

1. Prepare a blotting membrane covered with HRP-conjugated antibody.
2. Prepare 5mL of deionized water, add these solutions in the kit and prepare the DAB substrate solution.
   - Add 5 drops of DAB solution (about 140 µL)
   - Add 2 drops of Hydrogen Peroxide solution (about 80 µL)
   - Add 2 drops of 1.5M Tris-HCl buffer (about 80 µL)
3. Soak the membrane in the DAB substrate solution and incubate until the membrane is colored.
4. Add deionized water and stop the coloring reaction.

Figure.
An example of Western blotting by the above method

M : molecular weight marker
1 : Target protein C

M 1

Related Products

Sodium Hydroxide (1mol/L in Water)	500mL	[S0542]
Hydrochloric Acid (1mol/L)	500mL	[H1202]
Horseradish Peroxidase Maleimide Conjugate (0.5mg×3)	1set	[H1621]
Horseradish Peroxidase NHS Ester Conjugate (0.2mg×3)	1set	[H1746]
Anti-6xHis Monoclonal Antibody (6A12) HRP Conjugate	0.05mg/vial	[A3075]
Anti-Protein A Chicken Polyclonal Antibody HRP Conjugate	0.05mg/vial	[A3187]
Anti-αGal Chicken Polyclonal Antibody HRP Conjugate	0.05mg/vial	[A3195]
Anti-NeuGc Polyclonal Antibody HRP Conjugate	0.05mg/vial	[A3397]
Goat Anti-Mouse IgG HRP Conjugate	0.1mg/vial	[G0407]
Goat Anti-Mouse IgM HRP Conjugate	0.1mg/vial	[G0417]
Goat Anti-Rabbit IgG HRP Conjugate	0.1mg/vial	[G0418]
Sheep Anti-Chicken IgY HRP Conjugate	0.1mg/vial	[S0999]
Protein A HRP Conjugate	0.2mg/vial	[P2466]
Streptavidin HRP Conjugate	0.1mg/vial	[S0972]

Soluble Substrates (for ELISA etc.)

For such as ELISA, substrates generating soluble dyes with peroxidase.

AzBTS (= 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid Ammonium Salt))	1g	[A2166]
OPD·2HCl (= 1,2-Phenylenediamine Dihydrochloride)	1g	[P1144]
OPD (= 1,2-Phenylenediamine)	1g / 5g	[P1805]
TMB (= 3,3',5,5'-Tetramethylbenzidine)	1g / 5g	[T2573]

Soluble Substrates (for determining H_2O_2)

Substrates generating soluble dyes for determining hydrogen peroxidase (H_2O_2) by various enzyme reactions.

4-AA·2HCl (= 4-Aminoantipyrine Hydrochloride)	5g / 25g	[A0257]
4-AA (= 4-Aminoantipyrine)	1g / 5g	[A2254]
5-ASA (= 5-Aminosalicylic Acid) *1	5g / 25g	[A2291]
DCHBS (= 3,5-Dichloro-2-hydroxybenzenesulfonic Acid Sodium Salt) *1	5g / 25g	[D1928]
2,4-DCP (= 2,4-Dichlorophenol) *1	1g / 5g	[D3865]
DMA (= *N*,*N*-Dimethylaniline) *1	1g / 5g	[D3866]
DMT (= *N*,*N*-Diethyl-*m*-toluidine) *1	1g / 5g	[D3868]
TOOS (= Sodium 3-[Ethyl(*m*-tolyl)amino]-2-hydroxy-1-propanesulfonate) *1	1g / 5g	[S0805]
ALPS (= Sodium 3-(*N*-Ethylanilino)propanesulfonate) *1	200mg / 1g	[S0817]
ADOS (= Sodium 3-(*N*-Ethyl-3-methoxyanilino)-2-hydroxy-1-propanesulfonate) *1	200mg / 1g	[S0826]
HDAOS (= *N*-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline Sodium Salt) *1	200mg	[S0827]
MBTH·HCl (= 3-Methyl-2-benzothiazolinonehydrazone Hydrochloride)	1g / 5g	[M2155]

*1 : Used together with A2254 (or A0257)

Precipitate Substrates

For such as immunohistochemical staining or immunoblotting, substrates arising precipitate products with peroxidase.

AEC (= 3-Amino-9-ethylcarbazole)	1g / 5g	[A2167]
4-CN (= 4-Chloro-1-naphthol)	1g / 5g	[C2291]
DAB (= 3,3'-Diaminobenzidine)	1g / 5g	[D3756]
DAB·4HCl Hydrate (= 3,3'-Diaminobenzidine Tetrahydrochloride Hydrate)	1g / 5g	[D3757]
***o*-Dianisidine** *2	1g / 5g	[D3864]
***o*-Dianisidine Dihydrochloride** *2	1g / 5g	[D3893]
DMPD·2HCl (= *N*,*N*-Dimethyl-1,4-phenylenediamine Dihydrochloride) *3	1g / 5g	[D3931]
1-Naphthol *3	1g / 5g	[N0864]

*2 : By combinating N0864 and D3931 *3 : Used together with C2291

For β-Galactosidase

Generating Insoluble Dyes

X-Gal 200mg / 1g [B3201]
(= 5-Bromo-4-chloro-3-indolyl β-D-Galactopyranoside) Blue

Bluo-Gal 20mg / 100mg [B3470]
(= 5-Bromo-3-indolyl β-D-Galactopyranoside) Deep blue

Salmon-Gal 20mg / 100mg [C2371]
(= 6-Chloro-3-indolyl β-D-Galactopyranoside) Light purplish red

Generating Soluble Dyes

ONPG 1g / 5g / 25g [N0418]
(= 2-Nitrophenyl β-D-Galactopyranoside) Yellow

PNPG 1g / 5g [N0616]
(= 4-Nitrophenyl β-D-Galactopyranoside) Yellow

For β-Glucuronidase

X-Gluc CHA Salt 10mg / 100mg [B3620]
(= 5-Bromo-4-chloro-3-indolyl β-D-Glucuronide Cyclohexylammonium Salt) Blue

X-Gluc Sodium Salt 10mg / 100mg [B3621]
(= 5-Bromo-4-chloro-3-indolyl β-D-Glucuronide Sodium Salt) Blue

For Luciferase

D-(-)-Luciferin 10mg / 50mg [A5030]

Every reagent in the category “Substrate” is manufactured for biochemical research.

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