SUBSTRATES

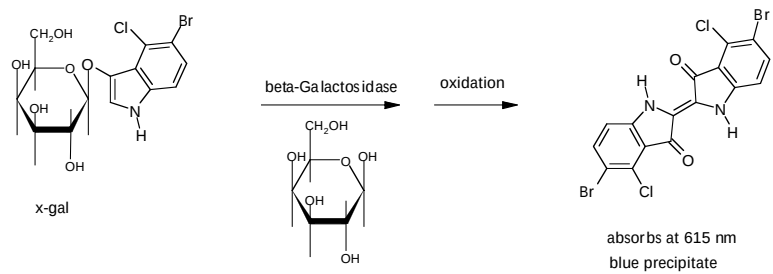
❶ Chromogenic Substrates

Indoxyl Substrates

e.g. 5-Bromo-4-chloro-3-indoxyl-beta-D-galactopyranoside (X-gal, x-b-D-gal)

The same basic reaction applies to the following chromogenic indigo substrate derivatives:

Substrates	color of precipitate	absorption lmax [nm]
X	blue	615
Y	blue	680
Magenta	magenta	565
Salmon	salmon	540
Iodo	purple	575
Green	green	665



BLUE I

Substrates for Glycosidases

Code	Product	Synonym
34048	X-beta-D-glucosaminide	5-Bromo-4-chloro-3-indoxyl-N-acetyl-beta-D-glucosaminide
34049	X-beta-D-cellobioside	5-Bromo-4-chloro-3-indoxyl-beta-D-cellobioside
34050	X-alpha-D-gal	5-Bromo-4-chloro-3-indoxyl-alpha-D-galactopyranoside
22777	X-gal, X-beta-D-gal	5-Bromo-4-chloro-3-indoxyl-beta-D-galactopyranoside
34051	X-alpha-D-glc	5-Bromo-4-chloro-3-indoxyl-alpha-D-glucopyranoside
22872	X-beta-D-glc	5-Bromo-4-chloro-3-indoxyl-beta-D-glucopyranoside
33701	X-beta-D-glcUA.CHX	5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt
33688	X-beta-D-glcUA.Na	5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, Na salt
34052	X-beta-D-xyl	5-Bromo-4-chloro-3-indoxyl-beta-D-xylopyranoside

Substrates for Esterases

Code	Product	Synonym
34053	X-palmitate	5-Bromo-4-chloro-3-indoxyl palmitate

Substrates for Phosphatases

Code	Product	Synonym
34055	X-phos.p-tol	5-Bromo-4-chloro-3-indoxyl phosphate, p-toluidine salt
33723	X-phos.K	5-Bromo-4-chloro-3-indoxyl phosphate, potassium salt

BLUE II

Substrates for Glycosidases

Code	Product	Synonym
33687	Y-beta-D-gal	3-Indoxyl-beta-D-galactopyranoside
33717	Y-beta-D-glc	3-Indoxyl-beta-D-glucopyranoside
33711	Y-beta-D-glcUA.CHX	3-Indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt
34031	Y-beta-D-glcUA.Na	3-Indoxyl-beta-D-glucuronic acid, sodium salt

Substrates for Phosphatases

Code	Product	Synonym
34032	Y-phos.diAMPD	3-Indoxyl phosphate, di(2-amino-2-methyl-1,3-propanediol) salt
33708	Y-phos.diNa	3-Indoxyl phosphate, disodium salt
34033	Y-phos.p-tol	3-indoxyl phosphate, p-touidine salt

Substrates for Sulfatases

Code	Product	Synonym
33706	Y-sulfate.K	3-indoxyl sulfate, potassium salt

MAGENTA

Substrates for Glycosidases

Code	Product	Synonym
34035	Magenta-beta-D-glucosaminide	5-Bromo-6-chloro-3-indoxyl-N-acetyl-beta-D-glucosaminide
33735	Magenta-beta-D-gal	5-Bromo-6-chloro-3-indoxyl-beta-D-galactopyranoside
34036	Magenta-beta-D-glc	5-Bromo-6-chloro-3-indoxyl-beta-D-glucopyranoside
33693	Magenta-beta-D-glcUA	5-Bromo-6-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt

Substrates for Esterases

Code	Product	Synonym
34037	Magenta-butyrate	5-Bromo-6-chloro-3-indoxyl butyrate
34038	Magenta-palmitate	5-Bromo-6-chloro-3-indoxyl palmitate

Substrate for Phosphatases

Code	Product	Synonym
33729	Magenta-phos.p-tol	5-Bromo-6-chloro-3-indoxyl phosphate, p-toluidine salt

Substrate for Sulfatases

Code	Product	Synonym
33724	Magenta-sulfate.K	5-Bromo-6-chloro-3-indoxyl sulfate, potassium salt

For prices, see alphabetical section of this catalogue

SALMON

Substrates for Glycosidases

Code	Product	Synonym
34039	Salmon-beta-D-gal	6-Chloro-3-indoxyl-beta-D-galactopyranoside
34040	Salmon-beta-D-glc	6-Chloro-3-indoxyl-beta-D-glucopyranoside
34041	Salmon-beta-D-glcUA.CHX	6-Chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt

Substrates for Phosphatases

Code	Product	Synonym
34045	Salmon-phos.p-tol	6-Chloro-3-indoxyl phosphate, p-toluidine salt

PURPLE

Code	Product	Synonym
34046	Iodo-beta-D-gal	5-Iodo-3-indoxyl-beta-D-galactopyranoside

GREEN

Code	Product	Synonym
34047	Green-beta-D-gal	N-Methylindoxyl-beta-D-galactopyranoside

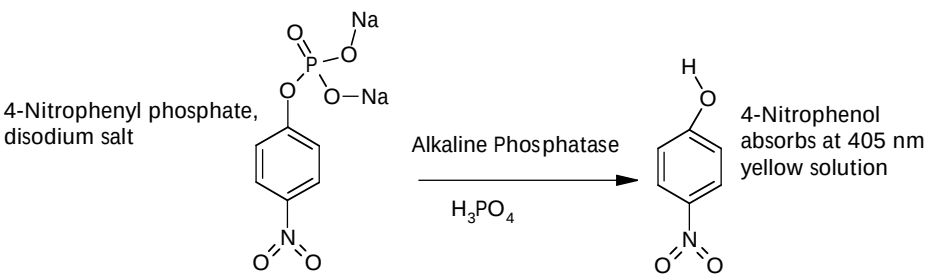
Oxidizing Agent

Code	Product	Synonym
41592	NBT	Nitrotertazolium blue chloride
15833	TNBT	Tetranitrobluetetrazolium chloride

2- and 4-Nitrophenyl (ONP and PNP) substrates

e.g. 4-Nitrophenyl phosphate, disodium salt (PNP-phos)

The same basic reaction applies for all 2- and 4-Nitrophenyl (ONP and PNP) substrates.



Substrates for Glycosidases

Code	Product	Synonym
33694	PNP-beta-D-galactosaminide	4-Nitrophenyl-N-acetyl-beta-D-galactosaminide
22939	ONP-alpha-D-glucosaminide	2-Nitrophenyl-N-acetyl-alpha-D-glucosaminide
34057	ONP-beta-D-glucosaminide	2-Nitrophenyl-N-acetyl-beta-D-glucosaminide
22940	PNP-alpha-D-glucosaminide	4-Nitrophenyl-N-acetyl-alpha-D-glucosaminide
22941	PNP-beta-D-glucosaminide	4-Nitrophenyl-N-acetyl-beta-D-glucosaminide
33710	PNP-beta-D-cellobiose	4-Nitrophenyl-beta-D-cellobioside
33685	PNP-beta-D-fucoside	4-Nitrophenyl-beta-D-fucopyranoside
12882	ONP-beta-D-gal	2-Nitrophenyl-beta-D-galactopyranoside
33733	PNP-alpha-D-gal	4-Nitrophenyl-alpha-D-galactopyranoside
22929	PNP-alpha-D-gal	4-Nitrophenyl-beta-D-galactopyranoside
33715	PNP-alpha-D-glc	4-Nitrophenyl-alpha-D-glucopyranoside
30311	PNP-beta-D-glc	4-Nitrophenyl-beta-D-glucopyranoside
33679	PNP-beta-D-glcUA	4-Nitrophenyl-beta-D-glucuronic acid
34061	PNP-beta-D-glcUA.Na	4-Nitrophenyl-beta-D-glucuronic acid, sodium salt
33696	PNP-alpha-D-maltoside	4-Nitrophenyl-alpha-D-maltopyranoside
33680	PNP-alpha-D-mannoside	4-Nitrophenyl-alpha-D-mannopyranoside

Substrates for Phosphatases

Code	Product	Synonym
22799	PNP-phos.diCHX	4-Nitrophenyl phosphate, dicyclohexylammonium salt
12886	PNP-phos.diNa	4-Nitrophenylphosphate, disodium salt

Horseradish Peroxidase / TMB System

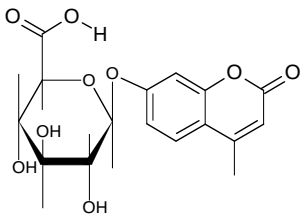
Code	Product	Synonym
22928	TMB	3,3',5,5'-tetramethylbenzidine, free base
22999	TMB.diHCl	3,3',5,5'-tetramethylbenzidine dihydrochloride

❷ Fluorogenic Substrates

Methylumbelliferyl (4-MU-) Substrates

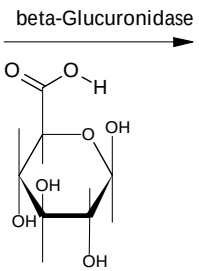
e.g. 4-Methylumbelliferyl-beta-D-glucuronic acid (MUG, 4-MU-b-D-glcUA)

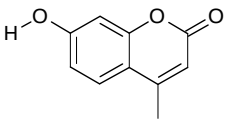
The same basic reaction applies for all 4-Methylumbelliferyl substrates.



4-Methylumbelliferyl-
beta-D-glucuronic acid

beta-Glucuronidase





Methylumbelliferone
emits at 460 nm
water soluble, fluorescent blue

For prices, see alphabetical section of this catalogue

Substrates for Glycosidases

Code	Product	Synonym
33712	4-MU-beta-D-galactosaminide	4-Methylumbelliferyl-N-acetyl-beta-D-galactosaminide
33714	4-MU-beta-D-glucosaminide	4-Methylumbelliferyl-N-acetyl-beta-D-glucosaminide
33716	4-MU-alpha-D-gal	4-Methylumbelliferyl-alpha-D-galactopyranoside
33721	4-MU-beta-D-gal	4-Methylumbelliferyl-beta-D-galactopyranoside
36699	4-MU-alpha-D-glc	4-Methylumbelliferyl-alpha-D-glucopyranoside
33702	4-MU-beta-D-glc	4-Methylumbelliferyl-beta-D-glucopyranoside
33722	MUG, 4-MU-beta-D-glcUA	4-Methylumbelliferyl-beta-D-glucuronic acid
33707	4-MU-alpha-D-mannoside	4-Methylumbelliferyl-alpha-D-mannopyranoside
33727	4-MU-beta-D-xyl	4-Methylumbelliferyl-beta-D-xylopyranoside

Substrates for Esterases

Code	Product	Synonym
34071	4-MU-nonanoate	4-Methylumbelliferyl nonanoate
33698	4-MU-palmitate	4-Methylumbelliferyl palmitate

Substrates for Phosphatases

Code	Product	Synonym
41504	4-MU-phos	4-Methylumbelliferyl phosphate, free acid
34073 salt	4-MU-phos.diAMPD	4-Methylumbelliferyl phosphate, di(2-amino-2-methyl-1,3 propanediol)
34074	4-MU-phos.diCHX	4-Methylumbelliferyl phosphate, dicyclohexylammonium salt
33704	4-MU-phos.diNa	4-Methylumbelliferyl phosphate, disodium salt

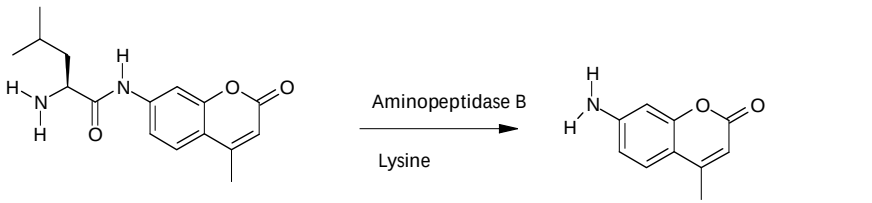
Substrate for Sulfatases

Code	Product	Synonym
34074	4-MU-sulfate.K	4-Methylumbelliferylsulfate, potassium salt

7-Amido-4-methylcoumarin (AMC) Substrates

e.g. Lysine 7-Amido-methylcoumarin (H-lys-AMC)

The same basic reaction applies for all 7-Amido-4-methylcoumarin (AMC) substrates.



Lysine 7-amido-4-methylcoumarin

7-Amino-4-methylcoumarin
water soluble, fluorescent blue

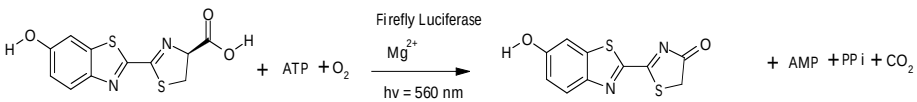
Code	Product	Synonym
34077	Ac-Lys-AMC.AcOH	N-Acetyl-L-lysine 7-amido-4-methylcoumarin, acetate salt
33737	H-Ala-AMC.TFA	L-Alanine 7-amido-methylcoumarin, trifluoroacetate salt
34078	H-Arg-Arg-AMC.HCl	L-Arginyl-L-arginine 7-amido-4-methylcoumarin hydrochloride
33692	H-Asp-AMC	L-Aspartic acid 7-amido-4-methylcoumarin
34079	N-CBZ-Gly-Pro-AMC	N-CBZ-Glycyl-L-proline 7-amido-4-methylcoumarin
34080	H-Cit-AMC.HBr	L-Citrulline 7-amido-4-methylcoumarin hydrobromide
34081	H-Cit-AMC.TFA	L-Citrulline 7-amido-4-methylcoumarin, trifluoroacetate salt
33732	H-Glu-AMC	gamma-L-Glutamic acid 7-amido-4-methylcoumarin
33681	H-Gly-AMC.HBr	L-Glycine 7-amido-4-methylcoumarin hydrobromide
34082	H-Gly-Gly-AMC.HCl	Glycyl-glycine 7-amido-4-methylcoumarin hydrochloride
34083	Gly-Phe-AMC	Glycyl-L-phenylalanine 7-amido-4-methylcoumarin
34084	Gly-Pro-AMC.HBr	Glycyl-L-proline 7-amido-4-methylcoumarin hydrobromide
34085	H-His-AMC	L-Histidine 7-amido-4-methylcoumarin
34086	H-Hyp-AMC	L-Hydroxyproline 7-amido-4-methylcoumarin
34087	H-Ile-AMC.TFA	L-Isoleucine 7-amido-4-methylcoumarin, trifluoroacetate salt
30310	H-Leu-AMC	L-Leucine 7-amido-4-methylcoumarin
33728	H-Leu-AMC.HCl	L-Leucine 7-amido-4-methylcoumarin hydrochloride
34088	H-Lys-AMC.AcOH	L-Lysine 7-amido-4-methylcoumarin, acetate salt
34089	H-Met-AMC.AcOH	L-Methionine 7-amido-4-methylcoumarin, acetate salt
34090	H-Orn-AMC.Cab	L-Ornithine 7-amido-4-methylcoumarin, carbonate salt
34091	H-Phe-AMC.TFA	L-Phenylalanine 7-amido-4-methylcoumarin, trifluoroacetate salt
33682	H-Pro-AMC.HBr	L-Proline 7-amido-4-methylcoumarin hydrobromide
33726	H-Pyr-AMC	L-Pyroglutamic acid 7-amido-4-methylcoumarin
33683	H-Ser-AMC.HCl	L-Serine 7-amido-4-methylcoumarin hydrochloride
34092	H-Ser-Tyr-AMC	L-Seryl-L-tyrosine 7-amido-4-methylcoumarin
34094	H-Trp-AMC	L-Tyrosine 7-amido-4-methylcoumarin
34095	H-Val-AMC	L-Valine 7-amido-4-methylcoumarin
34096	H-Val-AMC.TFA	L-Valine 7-amido-4-methylcoumarin trifluoroacetate salt

❸ Bioluminescent Substrates

Firefly Luciferase Substrates

e.g. Luciferin Firefly, free acid, synth.

The same basic reaction occurs when Luciferase is added to the system or Luciferase is expressed by a reporter gene.



Luciferin — Oxyluciferin

Code	Product
33705	Luciferin Firefly, free acid (synth.)
33678	Luciferin Firefly, sodium salt (synth.)
34097	Luciferase, recombinant

Apoaequorin- and Renilla Luciferase-Substrate: Coelenterazine

The different wavelengths compared to applications with Firefly Luciferase make double reporter gene applications possible.

Code	Product
34098	Coelenterazine
34099	2-(4-Dehydroxy)coelenterazine

4 Enzyme/Cross Reference List

Glycosidases

Enzyme	Code	Substrate
beta-Cellobiosidase	34049	5-Bromo-4-chloro-3-indoxyl-beta-D-cellobioside
	33710	4-Nitrophenyl-beta-D-cellobioside
beta-D-fucosidase	34102	4-Methylumbelliferyl-beta-D-fucopyranoside
	33685	4-Nitrophenyl-beta-D-fucopyranoside
beta-L-Fucosidase	34103	4-Methylumbelliferyl-beta-L-fucopyranoside
beta-Galactosaminidase	33712	4-Methylumbelliferyl-N-acetyl-beta-D-galactosaminide
	33694	4-Nitrophenyl-N-acetyl-beta-D-galactosaminide
alpha-Galactosidase	34050	5-Bromo-4-chloro-3-indoxyl-alpha-D-galactopyranoside
	33716	4-Methylumbelliferyl-alpha-D-galactopyranoside
	33733	4-Nitrophenyl-alpha-D-galactopyranoside
beta-Galactosidase	22777	5-Bromo-4-chloro-3-indoxyl-beta-D-galactopyranoside
	33735	5-Bromo-6-chloro-3-indoxyl-beta-D-galactopyranoside
	34039	6-Chloro-3-indoxyl-beta-D-galactopyranoside
	34108	8-Hydroxyquinoline-beta-D-galactopyranoside
	33687	3-Indoxyl-beta-D-galactopyranoside
	34046	5-Iodo-3-indoxyl-beta-D-galactopyranoside
	34047	N-Methylindoxyl-beta-D-galactopyranoside
	33721	4-Methylumbelliferyl-beta-D-galactopyranoside
	22942	2-Naphthyl-beta-D-galactopyranoside
	12882	2-Nitrophenyl-beta-D-galactopyranoside
	22929	4-Nitrophenyl-beta-D-galactopyranoside
alpha-Glucosaminidase	22939	2-Nitrophenyl-N-acetyl-alpha-D-glucosaminide
	22940	4-Nitrophenyl-N-acetyl-alpha-D-glucosaminide

Enzyme	Code	Substrate
beta-Glucosaminidase	34048	5-Bromo-4-chloro-3-indoxyl-N-acetyl-beta-D-glucosaminide
	34035	5-Bromo-6-chloro-3-indoxyl-N-acetyl-beta-D-glucosaminide
	33714	4-Methylumbelliferyl-N-acetyl-beta-D-glucosaminide
	34057	2-Nitrophenyl-N-acetyl-beta-D-glucosaminide
	22941	4-Nitrophenyl-N-acetyl-beta-D-glucosaminide
alpha-Glucosidase	34051	5-Bromo-4-chloro-3-indoxyl-alpha-D-glucopyranoside
	36699	4-Methylumbelliferyl-alpha-D-glucopyranoside
	33715	4-Nitrophenyl-alpha-D-glucopyranoside
beta-Glucosidase	22872	5-Bromo-4-chloro-3-indoxyl-beta-D-glucopyranoside
	34036	5-Bromo-6-chloro-3-indoxyl-beta-D-glucopyranoside
	34040	6-Chloro-3-indoxyl-beta-D-glucopyranoside
	33717	3-Indoxyl-beta-D-glucopyranoside
	33702	4-Methylumbelliferyl-beta-D-glucopyranoside
	30311	4-Nitrophenyl-beta-D-glucopyranoside
	30302	Phenyl-beta-D-galactopyranoside
	27082	Phenyl-beta-D-glucopyranoside
beta-Gluconidase	33701	5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, CHX salt
	33693	5-Bromo-6-chloro-3-indoxyl-beta-D-glucuronic acid, CHX salt
	33688	5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, sodium salt
	34041	6-Chloro-3-indoxyl-beta-D-glucuronic acid, CHX salt
	34113	8-Hydroxyquinoline-beta-D-glucuronide
	33711	3-Indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt
	34031	3-Indoxyl-beta-D-glucuronic acid, sodium salt
	33722	4-Methylumbelliferyl-beta-D-glucuronic acid
	33679	4-Nitrophenyl-beta-D-glucuronic acid
	34061	4-Nitrophenyl-beta-D-glucuronic acid, sodium salt
	33697	Phenyl-beta-D-glucuronic acid
alpha-Maltosidase	33696	4-Nitrophenyl-beta-D-maltopyranoside
alpha-Mannosidase	33707	4-Methylumbelliferyl-alpha-D-mannopyranoside
	33680	4-Nitrophenyl-alpha-D-mannopyranoside
beta-Xylosidase	34052	5-Bromo-4-chloro-3-indoxyl-beta-D-xylopyranoside
	33727	4-Methylumbelliferyl-beta-D-xylopyranoside

Esterases

Enzyme	Code	Substrate
Cholinesterase	34116	S-butyrylthiocholine iodide
Esterase (misc.)	34117	5-Bromo-4-chloro-3-indoxyl-3-acetate
	34037	5-Bromo-6-chloro-3-indoxyl butyrate
	34119	5-Bromo-6-chloro-3-indoxyl caprylate
	34053	5-Bromo-4-chloro-3-indoxyl palmitate
	34038	5-Bromo-6-chloro-3-indoxyl palmitate
	15666	3-Indoxyl-3-acetate
	34071	4-Methylumbelliferyl nonanoate
	33698	4-Methylumbelliferyl palmitate

For prices, see alphabetical section of this catalogue

Phosphatases

Enzyme	Code	Substrate
Alkaline Phosphatase	34055	5-Bromo-4-chloro-3-indoxyl phosphate, p-toluidine salt
	33729	5-Bromo-6-chloro-3-indoxyl phosphate, p-toluidine salt
	34045	6-Chloro-3-indoxyl phosphate, p-toluidine salt
	34032	3-Indoxyl phosphate, di(2-amino-2-methyl-1,3-propanediol) salt
	33708	3-Indoxyl phosphate, disodium salt
	34033	3-Indoxyl phosphate, p-toluidine salt
	41504	4-Methylumbelliferyl phosphate, free acid
	34073	4-Methylumbelliferyl phosphate, diAMPD salt
	34074	4-Methylumbelliferyl phosphate, dicyclohexylammonium salt
	33704	4-Methylumbelliferyl phosphate, disodium salt trihydrate
Phosphatase (misc.)	33723	5-Bromo-4-chloro-3-indoxyl phosphate, p-tol salt
	34121	1-Naphthyl phosphate, calcium salt
	22689	1-Naphthyl phosphate, sodium salt
	22799	4-Nitrophenyl phosphate, dicyclohexylammonium salt
	12886	4-Nitrophenyl phosphate, disodium salt
	34123	Phenolphtalein diphosphate, tetrasodium salt

Peptidases

Enzyme	Code	Substrate
g-Glutamyl Transpeptidase	33732	gamma-L-Glutamic acid 7-amido-4-methylcoumarin
	22871	L-gamma-glutamyl-4-nitroanilide monohydrate
Pyroglutamyl Peptidase	33726	L-Pyroglutamic acid 7-amido-4-methylcoumarin
Peptidase (misc.)	34077	N-Acetyl-L-lysine 7-amido-4-methylcoumarin, acetate salt
	33737	L-Alanine 7-amido-methylcoumarin, trifluoroacetate salt
	34078	L-Arginyl-L-arginine 7-amido-4-methylcoumarin hydrochloride
	33692	L-Aspartic acid 7-amido-4-methylcoumarin
	34079	N-CBZ-glycyl-L-proline 7-amido-4-methylcoumarin
	34080	L-Citrulline 7-amido-4-methylcoumarin hydrobromide
	34081	L-Citrulline 7-amido-4-methylcoumarin, trifluoroacetate salt
	33732	gamma-L-glutamic acid 7-amido-4-methylcoumarin
	33681	L-Glycine 7-amido-4-methylcoumarin hydrobromide
	34082	Glycyl-glycine 7-amido-4-methylcoumarin hydrochloride
	34083	Glycyl-L-phenylalanine 7-amido-4-methylcoumarin
	34084	Glycyl-L-proline 7-amido-4-methylcoumarin hydrobromide
	34085	L-Histidine 7-amido-4-methylcoumarin
	34086	L-Hydroxyproline 7-amido-4-methylcoumarin
	34087	L-Isoleucine 7-amido-4-methylcoumarin, trifluoroacetate salt
	30310	L-Leucine 7-amido-4-methylcoumarin
	33728	L-Leucine 7-amido-4-methylcoumarin hydrochloride
	34088	L-Lysine 7-amido-4-methylcoumarin, acetate salt
	34089	L-Methionine 7-amido-4-methylcoumarin, acetate salt

Enzyme	Code	Substrate
	34090	L-Ornithine 7-amido-4-methylcoumarin, carbonate salt
	34091	L-Phenylalanine 7-amido-4-methylcoumarin, TFA salt
	33682	L-Proline 7-amido-4-methylcoumarin hydrobromide
	33726	L-Pyroglutamic acid 7-amido-4-methylcoumarin
	33683	L-Serine 7-amido-4-methylcoumarin hydrochloride
	34092	L-Seryl-L-tyrosine 7-amido-4-methylcoumarin
	34094	L-Tyrosine 7-amido-4-methylcoumarin
	34095	L-Valine 7-amido-4-methylcoumarin
	34096	L-Valine 7-amido-4-methylcoumarin trifluoroacetate salt

Sulfatases

Enzyme	Code	Substrate
Arylsulfatase	33723	5-Bromo-4-chloro-3-indoxyl sulfate, potassium salt
	33724	5-Bromo-6-chloro-3-indoxyl sulfate, potassium salt
	33706	3-Indoxyl sulfate, potassium salt
	34074	4-Methylumbelliferylsulfate, potassium salt

5 How to use selected Enzyme Substrates

Substrate	Reaction Product Color
X-gal or X-glcUA	Blue
Magenta-gal or Magenta-glcUA	Magenta
Salmon-gal or Salmon-glcUA	Salmon

Preparation of the substrates: Solubilize in dimethylformamide (DMF) or dimethylsulfoxide (DMSO) at 25-50 mg/ml. This will then be the stock solution for further dilution into the final media. There is no need for sterilization of the stock solutions when they are made in DMF or DMSO.

Concentration of substrate in agar: The recommended starting concentrations of these substrates are listed below, but the concentration should be optimized for each application.

Substrate	Final Concentration in Media
X-gal or X-glcUA	50 µg/ml
Magenta-gal or Magenta-glcUA	100 µg/ml
Salmon-gal or Salmon-glcUA	150 µg/ml

Types of agar media: Minimal media are preferable, but other media will work. Due to catabolite repression of beta-Glucuronidase and beta-Galactosidase by glucose, it is preferable to use an alternative carbon source such as glycerol or succinate (7).

Adding substrates to the agar: Substrates should be added to the molten agar after it has cooled down to 80ºC. The X- and Magenta-substrates, but not the Salmon-substrates, can also be added to the media prior to autoclaving.

Stability of substrates in prepared agar plates: Prepared plates with these substrates have been found to be stable when stored for up to several months at 4ºC. In general the X-substrates are the most stable (< 6 months), followed by the Magenta-substrates (up to 3 months). The Salmon-substrates are the least stable. Additionally, the beta-Galactosidase substrates are less stable than the beta-Glucuronidase substrates.

pH effects: It has been observed that E. coli colonies growing on M9 minimal media containing either Magenta- or Salmon-substrates can show a diffuse halo of the colored product around the colony, rather than discrete localization of the color within the colony. The unadjusted pH of this medium is approximately pH 6.5. Lowering the pH to pH 6.0 resulted in even greater diffusion, whereas raising the pH to pH 7.0 or pH 7.5 resulted in the color being localized within the colonies.

Important considerations in using gus and lac substrates: Bacterial strains which possess the beta-Glucuronidase or beta-Galactosidase enzymes may or may not also possess the corresponding permease. The function of a specific permease is to transport the enzyme substrates across the bacterial membrane. For example, beta-Glucuronidase substrates are transported across the E. coli membrane via the glucuronide permease (3,5,7). The normal laboratory E. coli strain, K12 and its many derivatives, express beta-Glucuronidase, but lack a functional glucuronide permease. As a result, K12 strains are unable to actively take up gus substrates or inducers and appear to be gus negative in spite of the fact that they do contain the gus enzyme. However, almost all wild isolates of E. coli do have both an activity beta-Glucuronidase and an active glucuronide permease, and hence show strong gus activity (3,5,7). There is a similar permease responsible for transporting beta-Galactosidase substrates across the bacterial membrane, although this is not required for the uptake of indoxyl-galactoside substrates or the inducer IPTG (4).

Potential uses for these substrates in the detection and identification of bacteria: There are at least two broad areas of research in which there are many potential uses. The first is in environmental microbiology, where possession of beta-Galactosidase and/or beta-Glucuronidase activity is used as a diagnostic test for many bacteria (1,4 and see references in 7). Secondly, the gusA and lacZ genes encoding beta-Glucuronidase and beta-Galactosidase respectively are now being developed as microorganisms (3,6 and references in 7). (by Richard Jefferson, Ph.D. and Kate Wilson, Ph.D.)

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