

Lists of Enzyme Inhibitors and Substrates

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List of Inhibitors and Substrates for Various Proteases

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	Code	Compound	Quantity	Price:Yen	Page
ADAM-17					
Substrate					
	SDP-3818-PI	Abz-Leu-Ala-Gln-Ala-Val-Arg-Ser-Ser-Ser-Arg-A₂pr(Dnp)-NH₂	1 mg	15,000	317
	3226-v	MOCAC-Lys-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂	1 mg	8,000	221
ADAMTS-13					
Substrate					
	3224-s	FRETS-VWF73	0.1 mg	30,000	216
Aminopeptidases					
Inhibitor					
	4095	Amastatin (for Aminopeptidase A/PS and Leucyl Aminopeptidase)	25 mg★	99,300	182
	4148-v	Arphamenine A (for Aminopeptidase B)	0.5 mg	3,400	184
	4149	Arphamenine B (for Aminopeptidase B)	25 mg★	24,000	185
	4155	Ebelactone A (for N-Formylmethionyl Aminopeptidase)	25 mg	100,000	190
	4249-v	Leuhistin (for Aminopeptidase M)	0.5 mg	3,400	195
	4342-v	Phebestin (for Aminopeptidase N)	5 mg	10,000	197
Substrate					
	3147-v	Ala-MCA	5 mg	2,700	208
	3068	Ala-pNA	0.1 g★	2,100	208
	3091-v	Leu-MCA	5 mg	2,700	218
	3027	Leu-NH₂ • HCl	0.1 g★	1,900	218
	3014	Leu-pNA	0.1 g★	1,800	218
	3132-v	Lys-MCA	5 mg	3,600	219
	3149-v	Met-MCA	5 mg	3,500	219
	3148-v	Phe-MCA	5 mg	2,700	222
Angiotensin I Converting Enzyme					
Inhibitor					
	4009-v	Bradykinin-Potentiator B	0.5 mg	2,800	183(26)
	4010-v	Bradykinin-Potentiator C	0.5 mg	2,300	183(27)
	4097-v	Des-Pro²-Bradykinin	0.5 mg★	2,800	183(28)
	4190-v	Foroxymithine	0.5 mg★	6,500	192
Substrate					
	3126	Bz-Gly-Ala-Pro	0.1 g★	4,300	214
	3128	Bz-Gly-Gly-Gly	0.1 g★	2,900	214
	3064	Bz-Gly-His-Leu	0.1 g★	4,300	214
D-Aspartyl Endopeptidase					
Inhibitor					
	3223-v	Bz-Arg-His-D-Asp-CH₂Cl [Bz-RHd-CMK]	5 mg	20,000	186
Substrate					
	3222-v	Suc-D-Asp-MCA	5 mg	6,000	226

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

★ Other packaging is available.

List of Inhibitors and Substrates for Various Proteases (continued)

	Code	Compound	Quantity	Price:Yen	Page
Calpains					
Inhibitor					
	4096	E-64	25 mg*	11,400	189
	4320-v	E-64-c	5 mg	10,000	189
	4321-v	E-64-d (Proinhibitor)	5 mg	10,000	189
	3178-v	Z-Leu-Leu-H (aldehyde)	5 mg	4,000	200
Substrate					
	3104-v	Boc-Val-Leu-Lys-MCA	5 mg	5,700	212
	3120-v	Suc-Leu-Leu-Val-Tyr-MCA	5 mg	6,000	227
Carboxypeptidase A					
Substrate					
	3197-v	4-Methoxyphenylazoformyl-Phe [AAFP]	5 mg	3,000	219
Caspases					
Inhibitor					
	3221-v	Ac-Asp-Asn-Leu-Asp-H (aldehyde) (for Caspase-3)	5 mg	20,000	178
	3194-v	Ac-Asp-Gln-Thr-Asp-H (aldehyde) (for Caspase-7/3)	5 mg	25,000	178
	3172-v	Ac-Asp-Glu-Val-Asp-H (aldehyde) (for Caspase-3/7/8)	5 mg	20,000	178
	3192-v	Ac-Asp-Met-Gln-Asp-H (aldehyde) (for Caspase-3)	5 mg	30,000	179
	3196-v	Ac-Ile-Glu-Thr-Asp-H (aldehyde) (for Caspase-8/6, Granzyme B)	5 mg	20,000	179
	3199-v	Ac-Leu-Glu-His-Asp-H (aldehyde) (for Caspase-9)	5 mg	25,000	179
	3187-v	Ac-Trp-Glu-His-Asp-H (aldehyde) (for Caspase-1)	5 mg	20,000	180
	3180-v	Ac-Tyr-Val-Ala-Asp-CH₂Cl (for Caspases)	5 mg	20,000	180
	3165-v	Ac-Tyr-Val-Ala-Asp-H (aldehyde) (for Caspase-1)	5 mg	20,000	180
	3166-v	Ac-Tyr-Val-Lys-Asp-H (aldehyde) (for Caspase-1)	5 mg	20,000	181
	3204-v	Ac-Val-Asp-Val-Ala-Asp-H (aldehyde) (for Caspase-2)	5 mg	30,000	181
	3182-v	Ac-Val-Glu-Ile-Asp-H (aldehyde) (for Caspase-6)	5 mg	20,000	181
	3173-v	Biotinyl-Asp-Glu-Val-Asp-H (aldehyde) (for Caspase-3/7/8)	1 mg	10,000	185/239
	3174-v	Z-Asp-CH₂-DCB (for Caspases)	5 mg	15,000	199
	3189-v	Z-Glu-Lys(Biotinyl)-Asp-CH₂-DMB (for Caspases)	1 mg	10,000	199
	3188-v	Z-Val-Ala-Asp(OMe)-CH₂F (for Caspases)	1 mg	10,000	201
Substrate					
	3220-v	Ac-Asp-Asn-Leu-Asp-MCA (for Caspase-3)	5 mg	7,000	204
	3193-v	Ac-Asp-Gln-Thr-Asp-MCA (for Caspase-7/3)	5 mg	9,000	204
	3171-v	Ac-Asp-Glu-Val-Asp-MCA (for Caspase-3/7/8)	5 mg	7,000	205
	3195-v	Ac-Ile-Glu-Thr-Asp-MCA (for Caspase-8/6, Granzyme B)	5 mg	10,000	205
	3198-v	Ac-Leu-Glu-His-Asp-MCA (for Caspase-9)	5 mg	10,000	206
	3186-v	Ac-Trp-Glu-His-Asp-MCA (for Caspase-1/14)	5 mg	10,000	206
	3161-v	Ac-Tyr-Val-Ala-Asp-MCA (for Caspase-1)	5 mg	7,000	207
	3203-v	Ac-Val-Asp-Val-Ala-Asp-MCA (for Caspase-2)	5 mg	10,000	207
	3181-v	Ac-Val-Glu-Ile-Asp-MCA (for Caspase-6)	5 mg	7,000	207
	3184-v	MOCac-Asp-Glu-Val-Asp-Ala-Pro-Lys(Dnp)-NH₂ (for Caspase-3)	1 mg	15,000	220
	3183-v	MOCac-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-NH₂ (for Caspase-1)	1 mg	15,000	222

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

* Other packaging is available.

List of Inhibitors and Substrates for Various Proteases (continued)

Code	Compound	Quantity	Price:Yen	Page
Cathepsins and Thiol Proteases				
Inhibitor				
4062	Antipain (for Papain, Cathepsin A/B)	25 mg [*]	8,600	184
4322-v	CA-074 (for Cathepsin B)	5 mg	15,000	188
4323-v	CA-074 Me (Proinhibitor) (for Cathepsin B)	5 mg	15,000	188
4063	Chymostatin (for Papain, Cathepsin B/G)	25 mg [*]	15,300	186
4096	E-64 (for Thiol Proteases)	25 mg [*]	11,400	189
4320-v	E-64-c (for Cathepsin B/H/L)	5 mg	10,000	189
4321-v	E-64-d (Proinhibitor) (for Cathepsin B/H/L)	5 mg	10,000	189
4041	Leupeptin (for Papain, Cathepsin B)	25 mg [*]	5,700	195
4397	Pepstatin A (Purity: higher than 90% (HPLC)) (for Cathepsin D/E)	25 mg [*]	7,000	197
3175-v	Z-Leu-Leu-Leu-H (aldehyde) [MG-132] (for Cathepsin K)	5 mg	4,000	200
Substrate				
3113-v	Arg-MCA (for Cathepsin H)	5 mg	3,500	209
3057	Bz-L-Arg-pNA • HCl [L-BAPA] (for Papain)	0.1 g [*]	2,600	214
3119	Gly-Gly-Tyr-Arg (Affinity Ligand for Papain)	0.1 g [*]	5,800	217(192)
3200-v	MOCac-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH₂ (for Cathepsin D/E)	1 mg	16,000	220
3225-v	MOCac-Gly-Ser-Pro-Ala-Phe-Leu-Ala-Lys(Dnp)-D-Arg-NH₂ [KYS-1] (for Cathepsin E)	1 mg	10,000	220
3130	Pyr-Phe-Leu-pNA (for Thiol Proteases)	0.1 g [*]	4,800	224
3123-v	Z-Arg-Arg-MCA (for Cathepsin B)	5 mg	4,100	228
3208-v	Z-Gly-Pro-Arg-MCA (for Cathepsin K)	5 mg	5,000	229
3210-v	Z-Leu-Arg-MCA (for Cathepsin K/S/V)	5 mg	5,000	230
3095-v	Z-Phe-Arg-MCA (for Cathepsin B/L)	5 mg	3,500	231
3211-v	Z-Val-Val-Arg-MCA (for Cathepsin S/L)	5 mg	6,000	231
3018	Z-Glu-Tyr	0.1 g [*]	2,100	229
Chymotrypsin and Chymotrypsin-like Proteases				
Inhibitor				
4063	Chymostatin	25 mg [*]	15,300	186
3202-v	Ala-Ala-Phe-CH₂Cl [AAF-CMK]	5 mg	6,000	182
Substrate				
3154-v	Glt-Ala-Ala-Phe-MCA	5 mg	5,400	216
3114-v	Suc-Ala-Ala-Pro-Phe-MCA	5 mg	6,000	225
3120-v	Suc-Leu-Leu-Val-Tyr-MCA	5 mg	6,000	227
3006	Ac-Phe-OEt	0.1 g [*]	1,600	206
3034	Ac-Trp-OEt	0.1 g [*]	1,800	206
3009	Ac-Tyr-NH ₂	0.1 g [*]	1,700	206
3008	Ac-Tyr-OEt • H ₂ O	0.1 g [*]	1,700	207
3015	Bz-Tyr-pNA	0.1 g [*]	2,700	215
3010	Bz-Tyr-OEt	0.1 g [*]	1,600	214
3162-v	Suc-Ala-Leu-Pro-Phe-pNA	10 mg	10,000	225
3110-v	Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA	1 mg	5,400	226
3150-v	Suc-Ile-Ile-Trp-MCA	5 mg	6,000	226

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

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List of Inhibitors and Substrates for Various Proteases (continued)

	Code	Compound	Quantity	Price:Yen	Page
Coagulation Factors					
Inhibitor					
	4062	Antipain	25 mg★	8,600	184
	4041	Leupeptin	25 mg★	5,700	195
Substrate					
	3139-v	Boc-Asp(OBzl)-Pro-Arg-MCA (for α -Thrombin)	5 mg	5,000	209
	3136-v	Boc-Gln-Gly-Arg-MCA (for Factor XIIa)	5 mg	5,000	210
	3134-v	Boc-Glu(OBzl)-Ala-Arg-MCA (for Factor XIa)	5 mg	5,000	210
	3094-v	Boc-Ile-Glu-Gly-Arg-MCA (for Factor Xa)	5 mg	5,400	211
	3112-v	Boc-Leu-Ser-Thr-Arg-MCA (for Activated Protein C)	5 mg	6,000	212
	3125	Boc-Leu-Ser-Thr-Arg-pNA (for Activated Protein C)	25 mg★	20,000	212
	3106-v	Boc-Leu-Thr-Arg-MCA (for Factor VIIa-Tf)	5 mg	5,000	212
	3093-v	Boc-Val-Pro-Arg-MCA (for α -Thrombin)	5 mg	4,800	213
	3138-v	Z-Pyr-Gly-Arg-MCA (for Factor Xa)	5 mg	5,400	231
Collagenases / MMPs / Stromelysins					
Inhibitor					
	INH-3850-PI	TAPI-0	1 mg	25,000	316
	INH-3855-PI	TAPI-1	1 mg	25,000	316
	INH-3852-PI	TAPI-2	1 mg	25,000	316
Substrate					
	3226-v	MOCac-Lys-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂	1 mg	8,000	221
	3163-v	MOCac-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂	1 mg	6,000	221
	3168-v	MOCac-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH₂ [NFF-3]	1 mg	8,000	220
	3167-v	MOCac-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH₂ [NFF-2]	1 mg	8,000	220
	3087-v	Dnp-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg	2.5 mg	9,100	215
	3073-v	Dnp-Pro-Leu-Gly-Ile-Ala-Gly-Arg-NH ₂	2.5 mg	8,400	215
	3108-v	Suc-Gly-Pro-Leu-Gly-Pro-MCA	5 mg	6,000	226
	3029	Z-Gly-Pro-Leu-Gly-Pro • H ₂ O • AcOEt	0.1 g★	7,800	230
Dipeptidyl-Peptidases					
Inhibitor					
	IDP-3655-PI	Dab-Pip (for Dipeptidyl-Peptidase II)	5 mg	7,000	315
	4132	Diprotin A (for Dipeptidyl-Peptidase IV)	25 mg★	4,800	187
Substrate					
	3090-v	Gly-Pro-MCA (for X-Prolyl Dipeptidyl-Peptidase)	5 mg	3,000	217
	3074-v	Gly-Pro-pNA • Tos (for X-Prolyl Dipeptidyl-Peptidase)	10 mg	2,700	218
	3124-v	Lys-Ala-MCA (for Dipeptidyl-Peptidase II)	5 mg	3,600	219
Elastases					
Inhibitor					
	4243-v	Elafin (Human)	20 μ g	20,000	191
	4064	Elastatinal	25 mg★	16,700	191
Substrate					
New	3228-v	Pyr-Pro-Val-pNA	5 mg	6,000	222
	3133-v	Suc-Ala-Ala-Ala-MCA	5 mg	6,000	224
	3071	Suc-Ala-Ala-Ala-pNA [STANA]	0.1 g★	4,400	225
	3153-v	Suc(OMe)-Ala-Ala-Pro-Val-MCA	5 mg	6,000	224
	3129	Glt-Ala-Ala-Pro-Leu-pNA	0.1 g★	7,800	216
	3100-v	Suc-Ala-Pro-Ala-MCA	5 mg	6,000	225
	3118	Suc-Ala-Pro-Ala-pNA	0.1 g★	4,800	226

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

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List of Inhibitors and Substrates for Various Proteases (continued)

Code	Compound	Quantity	Price:Yen	Page
Furin and Carboxyl Side of Paired Basic Residue Cleaving Enzymes				
Substrate				
3122-v	Boc-Gln-Arg-Arg-MCA	5 mg	5,400	210
3159-v	Pyr-Arg-Thr-Lys-Arg-MCA	5 mg	6,500	223
3155-v	Boc-Arg-Val-Arg-Arg-MCA	5 mg	6,300	209
3142-v	Boc-Gly-Arg-Arg-MCA	5 mg	5,400	211
3143-v	Boc-Gly-Lys-Arg-MCA	5 mg	5,700	211
3140-v	Boc-Leu-Arg-Arg-MCA	5 mg	5,400	211
3141-v	Boc-Leu-Lys-Arg-MCA	5 mg	5,700	212
Gingipains				
Inhibitor				
4395-v	KYT-1 (for Arg-Gingipain)	1 mg	25,000	193
4396-v	KYT-36 (for Lys-Gingipain)	1 mg	25,000	193
Substrate				
3107-v	Boc-Phe-Ser-Arg-MCA (for Arg-Gingipain)	5 mg	5,000	212
3215-v	Z-His-Glu-Lys-MCA (for Lys-Gingipain)	5 mg	5,700	230
3095-v	Z-Phe-Arg-MCA (for Arg-Gingipain)	5 mg	3,500	231
γ-Glutamyl Transpeptidase				
Substrate				
3066	Glu(pNA)	0.1 g*	1,700	216
Hepatocyte Growth Factor-Activator (HGF-A)				
Substrate				
3185-v	Ac-Lys-Thr-Lys-Gln-Leu-Arg-MCA	5 mg	12,000	206
HIV-1 Protease				
Substrate				
4236-v	Ser-Gln-Asn-Tyr-Pro-Ile-Val	5 mg	15,000	224
Horseshoe Crab Clotting Enzyme				
Substrate				
3102-v	Boc-Leu-Gly-Arg-MCA	5 mg	5,000	211
Kallikreins				
Substrate				
3096-v	Pro-Phe-Arg-MCA	5 mg	5,400	223
3095-v	Z-Phe-Arg-MCA	5 mg	3,500	231
MMPs				
See Collagenases / MMPs / Stromelysins on page 159				
Neprilysin				
Substrate				
3127	Z-Ala-Ala-Leu-pNA	0.1 g*	4,400	228
Neutral Endopeptidase				
Inhibitor				
4082	Phosphoramidon	25 mg*	14,400	258
Substrate				
3111	Z-Gly-Gly-Leu-pNA	25 mg*	5,200	229

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

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List of Inhibitors and Substrates for Various Proteases (continued)

	Code	Compound	Quantity	Price:Yen	Page
Pepsin					
Inhibitor					
	4397	Pepstatin A (Purity : higher than 90% (HPLC))	25 mg★	7,000	197
Substrate					
	3216-v	MOCac-Ala-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(Dnp)-NH₂	1 mg	10,000	219
Peptidyl Prolyl <i>cis-trans</i> Isomerase (PPIase)					
Substrate					
	3162-v	Suc-Ala-Leu-Pro-Phe-pNA	10 mg	10,000	225
Plasmin					
Inhibitor					
	4041	Leupeptin	25 mg★	5,700	195
Substrate					
	3105-v	Boc-Glu-Lys-Lys-MCA	5 mg	6,000	210
	3104-v	Boc-Val-Leu-Lys-MCA	5 mg	5,700	212
Prolyl Endopeptidase					
Inhibitor					
	3214-v	SUAM-14746	5 mg	15,000	198
Substrate					
	3109-v	Suc-Gly-Pro-MCA	5 mg	4,100	226
Protein Kinase					
Inhibitor					
	4374-v	Lys-Lys-Lys-Leu-Arg-Arg-Gln-Glu-Ala-Phe-Asp-Ala-Tyr (for Calmodulin-Dependent Protein Kinase II)	0.5 mg	10,000	196
Substrate					
	4237-v	Pyr-Lys-Arg-Pro-Ser-Gln-Arg-Ser-Lys-Tyr-Leu (for Protein Kinase C)	5 mg	25,000	223
	4184-v	Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly [RR-SRC] (for Tyrosine Protein Kinase)	0.5 mg	7,200	208
Proteinase A					
Substrate					
	3216-v	MOCac-Ala-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(Dnp)-NH₂	1 mg	10,000	219
	3110-v	Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA	1 mg	5,400	226
Pyroglutamyl Peptidase					
Substrate					
	3079	Pyr-Ala	0.1 g★	2,700	223
	3101-v	Pyr-MCA	5 mg	3,500	223

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

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List of Inhibitors and Substrates for Various Proteases (continued)

	Code	Compound	Quantity	Price:Yen	Page
Renin					
Inhibitor					
	4397	Pepstatin A (Purity: higher than 90% (HPLC))	25 mg★	7,000	197
Substrate					
(New)	3229-v	Nma-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dnp)-D-Arg-D-Arg-NH₂	1 mg	15,000	222
	4133-v	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His	0.5 mg	4,300	209
	3110-v	Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA	1 mg	5,400	226
β-Secretase					
Inhibitor					
	4378-v	Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Sta-Val-Ala-Glu-Phe	1 mg	20,000	196
Substrate					
	3212-v	MOCac-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH₂	1 mg	15,000	221
γ-Secretase					
Inhibitor					
	4394-v	L-685,458	1 mg	30,000	194
	3219-v	(3,5-Difluorophenylacetyl)-Ala-Phg-OBu^t [DAPT]	5 mg	10,000	221
Substrate					
	3217-v	Nma-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH₂	1 mg	15,000	222
Signal Peptide Peptidase					
Inhibitor					
	3218-v	(Z-Leu-Leu-NHCH₂)₂CO [(Z-LL)₂Ketone]	5 mg	7,000	201
Stromelysins					
See Collagenases / MMPs / Stromelysins on page 159					
Subtilisin A					
Substrate					
	3127	Z-Ala-Ala-Leu-pNA	0.1 g★	4,400	228
u-PA (Urokinase)					
Inhibitor					
	4062	Antipain	25 mg★	8,600	184
Substrate					
	3058	Ac-Gly-Lys-OME [AGLME]	0.1 g★	2,200	205
	3097-v	Glt-Gly-Arg-MCA	5 mg	4,100	216
	3145-v	Pyr-Gly-Arg-MCA	5 mg	5,400	223
Transglutaminase					
Substrate					
	3190	Z-Gln-Gly	1 g★	15,000	228
Tripeptidyl Peptidase II					
Inhibitor					
	3202-v	Ala-Ala-Phe-CH₂Cl	5 mg	6,000	182
Substrate					
	3201-v	Ala-Ala-Phe-MCA	5 mg	4,000	208

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

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List of Inhibitors and Substrates for Various Proteases (continued)

	Code	Compound	Quantity	Price:Yen	Page
Trypsin and Trypsin-like Proteases					
Inhibitor					
	4062	Antipain	25 mg★	8,600	184
	4041	Leupeptin	25 mg★	5,700	195
Substrate					
	3135-v	Boc-Gln-Ala-Arg-MCA	5 mg	5,000	210
	3102-v	Boc-Leu-Gly-Arg-MCA	5 mg	5,000	211
	3107-v	Boc-Phe-Ser-Arg-MCA	5 mg	5,000	212
	3092-v	Bz-Arg-MCA	5 mg	3,500	213
	3001	Bz-Arg-OEt • HCl [BAEE]	1 g★	2,200	213
	3013	Bz-DL-Arg-pNA • HCl [DL-BAPA]	1 g★	3,800	213
	3057	Bz-L-Arg-pNA • HCl [L-BAPA]	1 g★	9,800	214
	3003	Tos-Arg-OMe • HCl [TAME]	5 g★	3,000	227
	3054	Tos-Lys-OMe • HCl	1 g★	3,600	227
	3078	Ac-Arg-OMe • HCl	1 g★	3,200	204
	3058	Ac-Gly-Lys-OMe	1 g★	8,300	205
	3002	Bz-Arg-NH ₂ • HCl • H ₂ O	1 g★	2,700	213
Enzymes in Ubiquitin-Proteasome System					
Inhibitor					
	4381-v	Epoxomicin (for Proteasome)	0.2 mg	20,000	191
	4368-v	Lactacystin (for Proteasome)	0.2 mg	20,000	194
	3207-v	Ubiquitin Aldehyde (for Deubiquitinating Enzyme)	50 µg	20,000	199
	3169-v	Z-Ile-Glu(OBu^t)-Ala-Leu-H (aldehyde) [PSI] (for Proteasome)	5 mg	6,000	200
	3175-v	Z-Leu-Leu-Leu-H (aldehyde) [MG-132] (for Proteasome)	5 mg	4,000	200
	3170-v	Z-Leu-Leu-Nva-H (aldehyde) [MG-115] (for Proteasome)	5 mg	4,000	201
Substrate					
	3140-v	Boc-Leu-Arg-Arg-MCA (for Proteasome)	5 mg	5,400	211
	3120-v	Suc-Leu-Leu-Val-Tyr-MCA (for Proteasome)	5 mg	6,000	227
	3176-v	Z-Leu-Arg-Gly-Gly-MCA (for Isopeptidase T)	5 mg	7,000	230
	3179-v	Z-Leu-Leu-Glu-MCA (for Proteasome)	5 mg	6,000	230
	3177-v	Z-Leu-Leu-Leu-MCA (for Proteasome)	5 mg	5,000	230
	3096-v	Pro-Phe-Arg-MCA (for Proteasome)	5 mg	5,400	223
	3160-v	Suc-Ala-Glu-MCA (for Proteasome)	5 mg	4,300	225
	3156-v	Z-Val-Lys-Met-MCA (for Proteasome)	5 mg	5,700	231
Vacuolar Processing Enzyme					
Substrate					
	3227-v	Ac-Glu-Ser-Glu-Asn-MCA	5 mg	10,000	205

For other enzymes, please refer to the section of the respective enzyme inhibitors and substrates.

★ Other packaging is available.

List of MCA-Substrates

List of MCA-Substrates

Code	Compound	Enzyme	Reference	Vial	Price:Yen	Page
3220-v	Ac-Asp-Asn-Leu-Asp-MCA	Caspase-3	91)	5 mg	7,000	204
3193-v	Ac-Asp-Gln-Thr-Asp-MCA	Caspase-7/3	65),66)	5 mg	9,000	204
3171-v	Ac-Asp-Glu-Val-Asp-MCA	Caspase-3/7/8	59),68)	5 mg	7,000	205
(New) 3227-v	Ac-Glu-Ser-Glu-Asn-MCA	Vacuolar Processing Enzyme	103),104),105)	5 mg	10,000	205
3195-v	Ac-Ile-Glu-Thr-Asp-MCA	Caspase-8/6 and Granzyme B	68)	5 mg	10,000	205
3198-v	Ac-Leu-Glu-His-Asp-MCA	Caspase-9	68)	5 mg	10,000	206
3185-v	Ac-Lys-Thr-Lys-Gln-Leu-Arg-MCA	Hepatocyte Growth Factor-Activator (HGF-A)	69)	5 mg	12,000	206
3186-v	Ac-Trp-Glu-His-Asp-MCA	Caspase-1/14	68),70)	5 mg	10,000	206
3161-v	Ac-Tyr-Val-Ala-Asp-MCA	Caspase-1	55)	5 mg	7,000	207
3203-v	Ac-Val-Asp-Val-Ala-Asp-MCA	Caspase-2	77)	5 mg	10,000	207
3181-v	Ac-Val-Glu-Ile-Asp-MCA	Caspase-6	71),72)	5 mg	7,000	207
3201-v	Ala-Ala-Phe-MCA	Tripeptidyl Peptidase II	74),75)	5 mg	4,000	208
3147-v	Ala-MCA	Aminopeptidase	22)	5 mg	2,700	208
3099-v	AMC	Reference Compound	1),2),3),8)	5 mg	2,000	208
3113-v	Arg-MCA	Cathepsin H	4),5),23),38),49)	5 mg	3,500	209
3144-v	Boc-Ala-Gly-Pro-Arg-MCA	rANP Precursor Processing Enzyme	41),47)	5 mg	5,700	209
3155-v	Boc-Arg-Val-Arg-Arg-MCA	Furin	54)	5 mg	6,300	209
3139-v	Boc-Asp(OBzl)-Pro-Arg-MCA	α -Thrombin	39)	5 mg	5,000	209
3135-v	Boc-Gln-Ala-Arg-MCA	Trypsin	39)	5 mg	5,000	210
3122-v	Boc-Gln-Arg-Arg-MCA	Carboxyl Side of Paired Basic Residue Cleaving Enzyme	39),40)	5 mg	5,400	210
3136-v	Boc-Gln-Gly-Arg-MCA	Factor XIIa	39)	5 mg	5,000	210
3105-v	Boc-Glu-Lys-Lys-MCA	Plasmin	6),7),27),39)	5 mg	6,000	210
3134-v	Boc-Glu(OBzl)-Ala-Arg-MCA	Factor XIa	39)	5 mg	5,000	210
3115-v	Boc-Glu(OBzl)-Gly-Arg-MCA		7),39)	5 mg	5,000	211
3142-v	Boc-Gly-Arg-Arg-MCA	Carboxyl Side of Paired Basic Residue Cleaving Enzyme	40)	5 mg	5,400	211
3143-v	Boc-Gly-Lys-Arg-MCA	Carboxyl Side of Paired Basic Residue Cleaving Enzyme	39),40)	5 mg	5,700	211
3094-v	Boc-Ile-Glu-Gly-Arg-MCA	Factor Xa	1),7),24),25),27)	5 mg	5,400	211
3140-v	Boc-Leu-Arg-Arg-MCA	Proteasome and Carboxyl Side of Paired Basic Residue Cleaving Enzyme	39),40),61)	5 mg	5,400	211
3102-v	Boc-Leu-Gly-Arg-MCA	Horseshoe Crab Clotting Enzyme	1),7),26),39)	5 mg	5,000	211
3141-v	Boc-Leu-Lys-Arg-MCA	Carboxyl Side of Paired Basic Residue Cleaving Enzyme	39),40)	5 mg	5,700	212
3112-v	Boc-Leu-Ser-Thr-Arg-MCA	Activated Protein C	7),13),39)	5 mg	6,000	212
3106-v	Boc-Leu-Thr-Arg-MCA	Factor VIIa-Tf	7),13),39)	5 mg	5,000	212
3107-v	Boc-Phe-Ser-Arg-MCA	Trypsin, Trypsin, and 73K Protease Arg-Gingipain	7),39),45),46)	5 mg	5,000	212
3104-v	Boc-Val-Leu-Lys-MCA	Plasmin and Calpain	6),7),27),39),62)	5 mg	5,700	212

List of MCA-Substrates (continued)

Code	Compound	Enzyme	Reference	Vial	Price:Yen	Page
3093-v	Boc-Val-Pro-Arg-MCA	α -Thrombin	1),7),24),25),27),29),39),40),45),50),53)	5 mg	4,800	213
3092-v	Bz-Arg-MCA	Trypsin	4)	5 mg	3,500	213
3154-v	Glt-Ala-Ala-Phe-MCA	Chymotrypsin		5 mg	5,400	216
3097-v	Glt-Gly-Arg-MCA	u-PA	1),7),27),29),35),39)	5 mg	4,100	216
3090-v	Gly-Pro-MCA	X-Prolyl Dipeptidyl-Aminopeptidase	16),17),49)	5 mg	3,000	217
3091-v	Leu-MCA	Aminopeptidase	8),9),37)	5 mg	2,700	218
3132-v	Lys-MCA	Aminopeptidase		5 mg	3,600	219
3124-v	Lys-Ala-MCA	Dipeptidyl-Aminopeptidase II	22)	5 mg	3,600	219
3149-v	Met-MCA	Aminopeptidase		5 mg	3,500	219
3148-v	Phe-MCA	Aminopeptidase	28)	5 mg	2,700	222
3096-v	Pro-Phe-Arg-MCA	Pancreatic / Urinary Kallikrein, and Proteasome	1),7),14),18),21),24),31),39),63)	5 mg	5,400	223
3159-v	Pyr-Arg-Thr-Lys-Arg-MCA	Furin		5 mg	6,500	223
3145-v	Pyr-Gly-Arg-MCA	t-PA and u-PA	39)	5 mg	5,400	223
3101-v	Pyr-MCA	Pyroglutamyl Peptidase	10),11)	5 mg	3,500	223
3153-v	Suc(OMe)-Ala-Ala-Pro-Val-MCA	Human Leukocyte / Porcine Pancreatic Elastase	48)	5 mg	6,000	224
3133-v	Suc-Ala-Ala-Ala-MCA	Elastase	28)	5 mg	6,000	224
3114-v	Suc-Ala-Ala-Pro-Phe-MCA	Chymotrypsin	33)	5 mg	6,000	225
3160-v	Suc-Ala-Glu-MCA	Ingensin and Proteasome	56)	5 mg	4,300	225
3100-v	Suc-Ala-Pro-Ala-MCA	Elastase	42)	5 mg	6,000	225
3110-v	Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA	Renin and Proteinase A	19),34)	1 mg	5,400	226
3222-v	Suc-D-Asp-MCA	D-Aspartyl Eudopeptidase	92)	5 mg	6,000	226
3108-v	Suc-Gly-Pro-Leu-Gly-Pro-MCA	Collagenase-like Peptidase	15)	5 mg	6,000	226
3109-v	Suc-Gly-Pro-MCA	Post-Proline Cleaving Enzyme, Prolyl Endopeptidase	20)	5 mg	4,100	226
3158-v	Suc-Ile-Ala-MCA	Amyloid A4 Generating Enzyme	49),52)	5 mg	4,300	226
3150-v	Suc-Ile-Ile-Trp-MCA			5 mg	6,000	226
3120-v	Suc-Leu-Leu-Val-Tyr-MCA	Chymotrypsin, Calpain, Ingensin, and Proteasome	33),43),44),51)62)	5 mg	6,000	227
3209-v	Z-Ala-Ala-Asn-MCA	Legumain	78),79),80),81)	5 mg	5,000	227
3123-v	Z-Arg-Arg-MCA	Cathepsin B	38),39)	5 mg	4,100	228
3208-v	Z-Gly-Pro-Arg-MCA	Cathepsin K	83),84)	5 mg	5,000	229
3215-v	Z-His-Glu-Lys-MCA	Lys-Gingipain	89)	5 mg	5,700	230
3210-v	Z-Leu-Arg-MCA	Cathepsin K/S/V	85),86)	5 mg	5,000	230
3176-v	Z-Leu-Arg-Gly-Gly-MCA	Isopeptidase T	64)	5 mg	7,000	230

List of MCA-Substrates (continued)

Code	Compound	Enzyme	Reference	Vial	Price:Yen	Page
3179-v	Z-Leu-Leu-Glu-MCA	Proteasome		5 mg	6,000	230
3177-v	Z-Leu-Leu-Leu-MCA	Proteasome	58)	5 mg	5,000	230
3095-v	Z-Phe-Arg-MCA	Plasma Kallikrein, Cathepsin B/L, and Arg-Gingipain	1),5),7),21),24),27),31),32),38),39),49)	5 mg	3,500	231
3138-v	Z-Pyr-Gly-Arg-MCA	Factor Xa	39)	5 mg	5,400	231
3156-v	Z-Val-Lys-Met-MCA	Amyloid A4 Generating Enzyme and Proteasome	49)	5 mg	5,700	231
3211-v	Z-Val-Val-Arg-MCA	Cathepsin S/L	87),88)	5 mg	6,000	231

List of MOCAC/Dnp type Substrates

List of MOCAC/Dnp type Substrates

Code	Compound	Enzyme	Reference	Vial	Price:Yen	Page
3216-v	MOCAC-Ala-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(Dnp)-NH₂	Proteinase A/Pepsin	90)	1 mg	10,000	219
3167-v	MOCAC-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH₂	Matrix Metalloproteinase	60)	1 mg	8,000	220
3168-v	MOCAC-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH₂	Matrix Metalloproteinase-3	60)	1 mg	8,000	220
3184-v	MOCAC-Asp-Glu-Val-Asp-Ala-Pro-Lys(Dnp)-NH₂	Caspase-3	73)	1 mg	15,000	220
3200-v	MOCAC-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH₂	Cathepsin D/E	76)	1 mg	16,000	220
3225-v	MOCAC-Gly-Ser-Pro-Ala-Phe-Leu-Ala-Lys(Dnp)-D-Arg-NH₂	Cathepsin E	93),94),101)	1 mg	10,000	220
3226-v	MOCAC-Lys-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂	Matrix Metalloproteinase, ADAM-17 and TACE	95),96),97)	1 mg	8,000	221
3164-s	MOCAC-Pro-Leu-Gly	Reference Compound for MOCAC-type Fluorescence-Quenching Substrate	57)	0.1 mg	2,000	221
3163-v	MOCAC-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂	Matrix Metalloproteinase	57)	1 mg	6,000	221
3212-v	MOCAC-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH₂	β -Secretase	82)	1 mg	15,000	221
3183-v	MOCAC-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-NH₂	Caspase-1	73)	1 mg	15,000	222

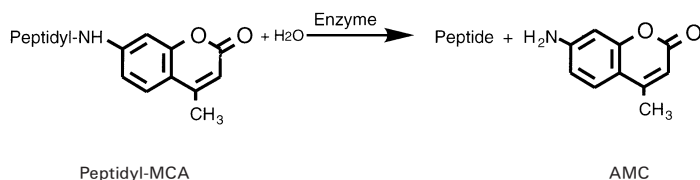
List of Nma/Dnp type Substrates

List of Nma/Dnp type Substrates

Code	Compound	Enzyme	Reference	Vial	Price:Yen	Page
3224-s	FRETs-VWF73	ADAMTS-13	98),99),102)	0.1 mg	30,000	216
3217-v	Nma-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH₂	γ -Secretase	100)	1 mg	15,000	222
(New) 3229-v	Nma-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dnp)-D-Arg-D-Arg-NH₂	Human Renin	106)	1 mg	15,000	222

Assay Methods Using Peptidyl-MCA Substrates (1)

Principle



The initial rate of increase in the AMC concentration can be monitored 1) fluorometrically at $\lambda_{\text{ex}} = 380 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$ (Fig. 1a) or 2) photometrically at 370 nm (Fig. 1b).

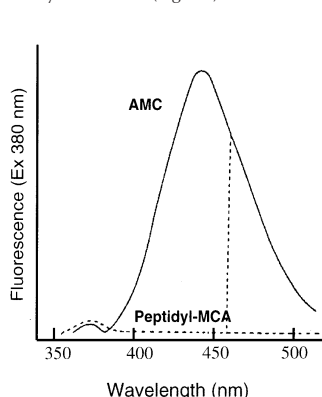


Fig. 1a Fluorescence Spectra

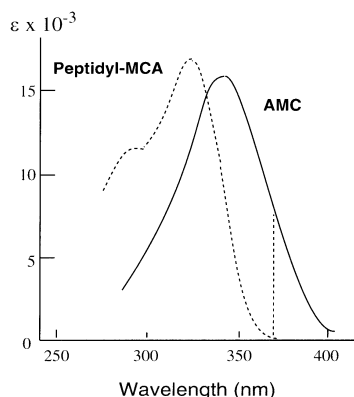


Fig. 1b UV-Absorption Spectra

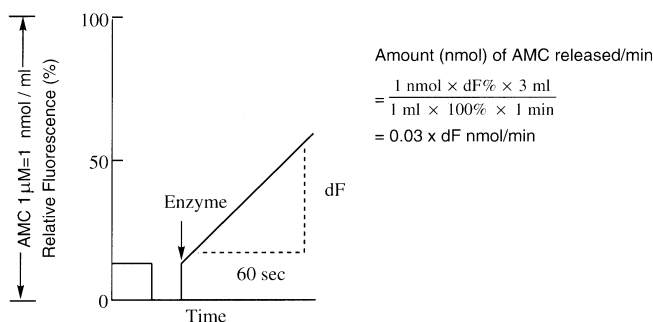
Reagents

- 1) Substrate stock solution: Vial, in DMSO at 10 mM
- 2) AMC stock solution: Content of vial (Code 3099-v AMC), in DMSO at 1 mM
- 3) Buffer
- 4) Enzyme solution

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the fluorometric method (initial-rate method).

- 1) Set a fluorescence spectrophotometer at $\lambda_{\text{ex}} = 380 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$ at 25 °C (1.0 Relative fluorescence unit at 10^{-6} M of AMC)
- 2) Pipette 2940 μl of buffer and 30 μl of substrate stock solution into the cuvette
- 3) Incubate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
- 4) Add 30 μl of enzyme solution
- 5) Record the increase of the fluorescence intensity for 3-4 min
- 6) Calculate the amount of AMC released using the following equation



* Photometric measurement can be carried out by the same procedure as that of the fluorometric method using a UV spectrophotometer. Set the wavelength at 370 nm (ϵ 7700).

Assay Methods Using Peptidyl-MCA Substrates (2)

(Measurement on an Auto-Fluorescence Spectrophotometer for Multiplate)

Reagents

- 1) Substrate stock solution: Content of vial, in DMSO at 10 mM
- 2) AMC standard solution: Content of vial (Code 3099-v AMC), in DMSO at 1 mM
- 3) Buffer
- 4) Enzyme solution

Instrument: Auto-fluorescence spectrophotometer

Selection of filter:

380 nm, 390 nm or 355 nm filter will be recommended for measurement.

In using 355 nm filter, observe caution for overlapping of fluorescence of the substrate itself.

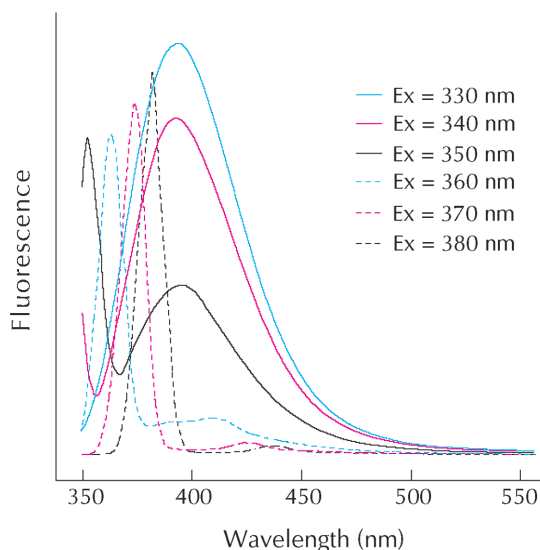


Fig. 2a Fluorescence Spectra of Peptidyl-MCA (Substrate)

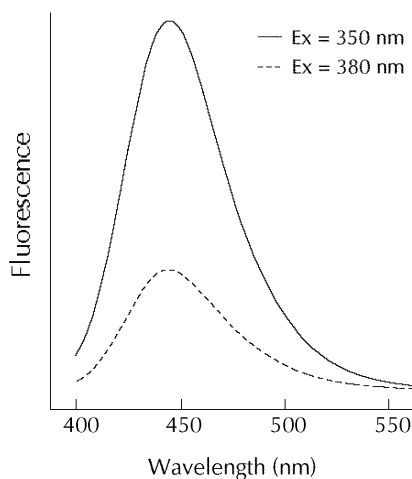


Fig. 2b Fluorescence Spectra of AMC (Product)

Procedure

Choose the proper conditions for the measurement, such as substrate, enzyme concentration and other reaction conditions, depending on the purpose of the experiment.

- 1) Set the auto-fluorescence spectrophotometer at $\lambda_{ex} = 380 \text{ nm}$, $\lambda_{em} = 460 \text{ nm}$ at 25°C
(1.0 Relative fluorescence unit at 10^{-6} M of AMC)
- 2) Pipette 160 μl of buffer and 20 μl of substrate solution in well for final concentration of 100 μM
- 3) Incubate the plate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
- 4) Take the multiplate out and add 20 μl of enzyme solution in each well
- 5) Mount the plate in the fluorescence spectrophotometer
- 6) Record the increase in fluorescence intensity for 30 min with a premixing time of 3 sec
- 7) Calculate the amount of released AMC

Assay Methods Using Peptidyl-MCA Substrates (3)

(Example for Caspase-7 using an Auto-fluorescence Spectrophotometer for Multiplate)

Reagents

- 1) Substrate stock solution: Content of vial (Code 3171-v Ac-DEVD-MCA), in DMSO at 10 mM
- 2) AMC standard solution: Content of vial (Code 3099-v AMC), in DMSO at 1 mM
- 3) Buffer: ICE standard buffer (100 mM HEPES-KOH, pH 7.5, 10% sucrose (w/v), 0.1% CHAPS (w/v), 10 mM DTT, 0.1 mg/ml ovalbumin)
- 4) Enzyme solution: rec Caspase-7 in buffer

Instrument: Fluoroskan Ascent (Labsystems)

This instrument can be used for both initial rate assay and end point assay

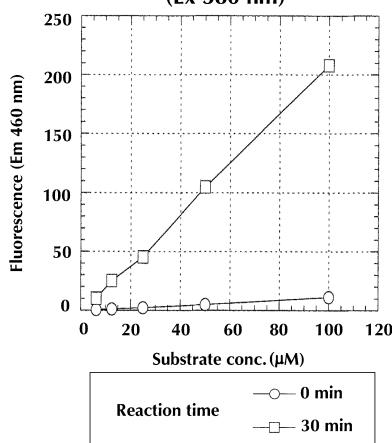
Procedure

a) Initial rate assay for 30 min at $\lambda_{ex} = 390 \text{ nm}$, $\lambda_{em} = 460 \text{ nm}$

b) End-point (30 min) assay at 5 different substrate concentrations at $\lambda_{ex} = 355 \text{ nm}$ or 380 nm , $\lambda_{em} = 460 \text{ nm}$

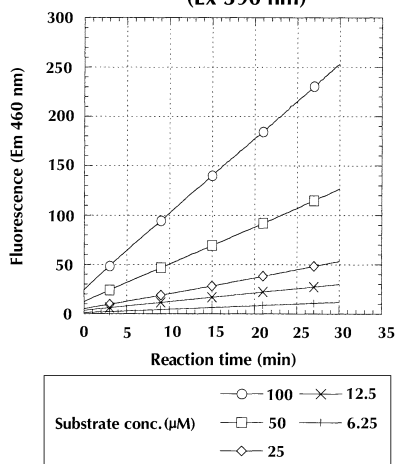
- 1) Set a fluorescence spectrophotometer at $\lambda_{ex} = 390 \text{ nm}$ (or 380 nm , 355 nm), $\lambda_{em} = 460 \text{ nm}$. Relative fluorescence is determined with 10^{-6} M of AMC at each condition
- 2) Substrate: Dilute the stock solution with the buffer ($\times 10$, $\times 20$, $\times 40$, $\times 80$, $\times 160$)
- 3) Caspase-7: Dissolve in buffer
- 4) Pipette $160 \mu\text{l}$ of buffer and $20 \mu\text{l}$ of substrate solutions (1 , $1/2$, $1/4$, $1/8$, $1/16 \text{ mM}$) in each well
- 5) Incubate in the fluorescence spectrophotometer for 3-4 min for temperature equilibration
- 6) Take the multiplate out and add $20 \mu\text{l}$ of enzyme solution to each well
- 7) Mount the plate in the fluorescence spectrophotometer
- 8) Calculate the amount of released AMC

b1) End-point assay
(Ex 380 nm)

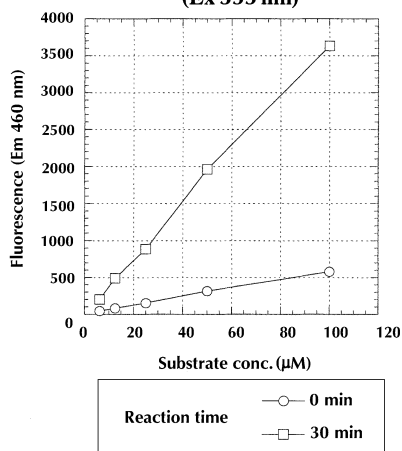


Results

a) Initial rate assay
(Ex 390 nm)



b2) End-point assay
(Ex 355 nm)



Assay Method Using MOCac/Dnp type Fluorescence-Quenching Substrates (Example using Code 3163-v MOCac-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂)

Principle

The highly fluorescent (7-methoxycoumarin-4-yl)acetyl (MOCac) group in the substrate such as Code 3163 is efficiently quenched by the 2,4-dinitrophenyl (Dnp) group. When metalloenzyme cleaves to the Gly-Leu bond, the fluorescence at $\lambda_{\text{ex}} = 328 \text{ nm}$ and $\lambda_{\text{em}} = 393 \text{ nm}$ increases 190-fold ⁽¹⁾.

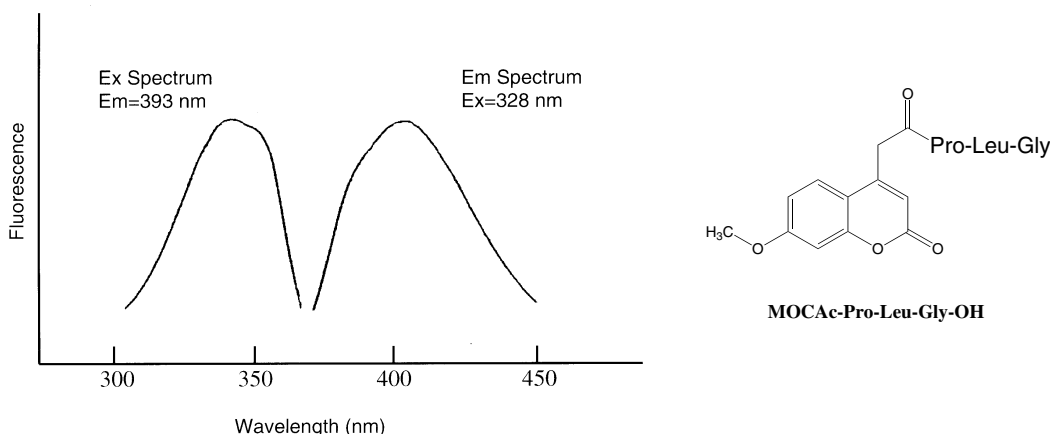


Fig. Fluorescence Spectra of MOCac-Pro-Leu-Gly-OH

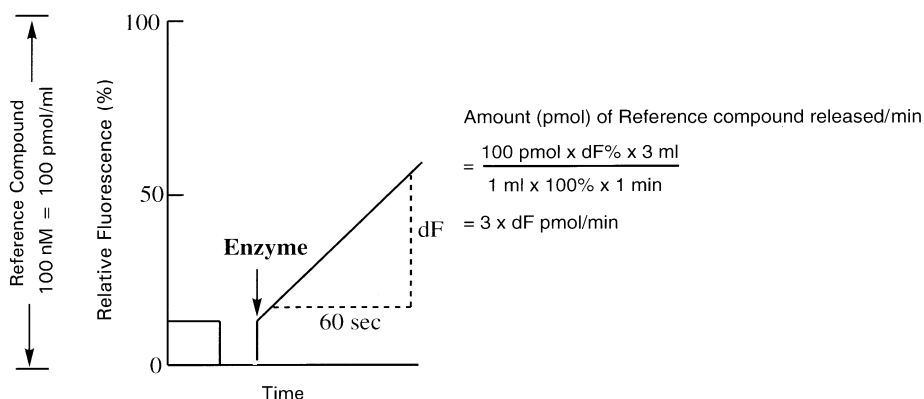
Reagents

- 1) Substrate stock solution: Code 3163-v in DMSO at $2 \times 10^{-4} \text{ M}$
- 2) MOCac-Pro-Leu-Gly (reference compound) stock solution: Code 3164-s in 10 ml of DMSO ($2 \times 10^{-5} \text{ M}$)
- 3) Buffer: 0.1 M-Tris • HCl, pH 7.5 containing 0.1 M NaCl, 10 mM CaCl₂ and 0.05% Brij-35
- 4) Enzyme solution

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the fluorometric method (initial-rate method).

- 1) Set a fluorescence spectrophotometer at $\lambda_{\text{ex}} = 328 \text{ nm}$ and $\lambda_{\text{em}} = 393 \text{ nm}$ at 25 °C (1.0 Relative fluorescence unit at 10^{-7} M of the reference compound)
- 2) Pipette 2900 μl of buffer and 50 μl of substrate stock solution into the cuvette
- 3) Incubate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
- 4) Add 50 μl of enzyme solution
- 5) Record the increase of the fluorescence intensity for 3-4 min
- 6) Calculate the amount of reference compound released using the following equation



(1) C.G. Knight, F. Willenbrock, and G. Murphy, *FEBS Lett.*, **296**, 263 (1992).

Assay Method Using Nma/Dnp type Fluorescence-Quenching Substrates

Principle

The highly fluorescent 2-(*N*-methylamino)benzoyl (Nma) group in the substrates such as Code 3217 and 3224 is efficiently quenched by the 2,4-dinitrophenyl (Dnp) group. When an enzyme of interest cleaves any peptide bond between Nma- and Dnp-containing amino acid residues in the substrate, the fluorescence at $\lambda_{ex} = 340 \text{ nm}$ and $\lambda_{em} = 440 \text{ nm}$ increases in proportion to the release of the Nma fluorophore from the internal Dnp quencher^{1,2}.

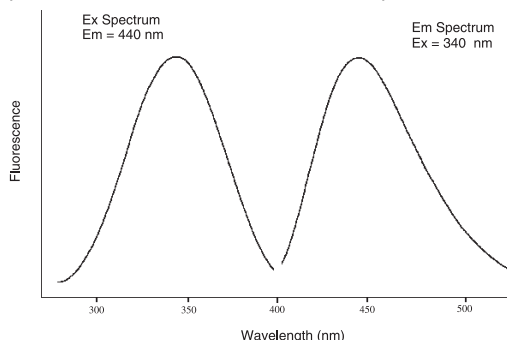


Fig. Fluorescence Spectra of a 1:1 mixture of D-Azpr(Nma)-Gly and Ala-Phe-Pro-Lys(Dnp)-D-Arg-D-Arg

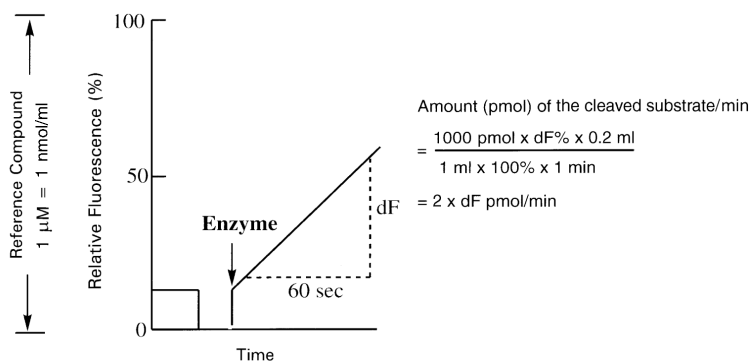
Reagents

- 1) Substrate stock solution: 100 μM -10 μM in an appropriate solvent
- 2) Reference compounds stock solution: a 1:1 mixture of two solutions of Code 3720-v and 3721-v, each of which is reconstituted by dissolving peptides in 0.5 ml of DMSO at the concentration of 2 mM (1 mM, each reference compound)
- 3) Enzyme solution: an enzyme of interest in an appropriate buffer
- 4) Buffer

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the fluorometric method (initial-rate method).

- 1) Set a fluorescence spectrometer at $\lambda_{ex} = 340 \text{ nm}$ and $\lambda_{em} = 440 \text{ nm}$ (1.0 Relative fluorescence unit at 1 μM of the reference compound)
- 2) Pipette 160 μl of buffer and 2-20 μl of substrate solution in well for final concentration of 1 μM .
- 3) Incubate in the fluorescence spectrophotometer for 3-4 min for temperature equilibration
- 4) Add 20 μl of enzyme solution prepared at an appropriate concentration
- 5) Record the increase of the fluorescence intensity
- 6) Calculate the amount of the cleaved substrate using the following equation



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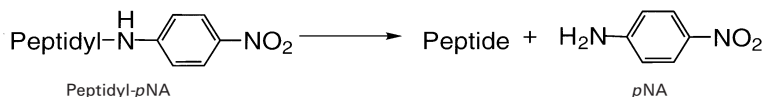
List of pNA-Substrates

Code	Compound	Enzyme	Bulk / Vial	Quantity	Price:Yen	Page
3068	Ala-pNA	Aminopeptidase	Bulk	0.1 g 1 g 5 g	2,100 6,400 27,200	208
3125	Boc-Leu-Ser-Thr-Arg-pNA	Activated Protein C	Bulk	25 mg 100 mg	20,000 60,000	212
3013	Bz-DL-Arg-pNA [DL-BAPA]	Trypsin-like Protease	Bulk	0.1 g 1 g 5 g	1,700 3,800 11,800	213
3057	Bz-L-Arg-pNA [L-BAPA]	Trypsin-like Protease, Papain	Bulk	0.1 g 1 g 5 g	2,600 9,800 37,000	214
3015	Bz-Tyr-pNA	Chymotrypsin	Bulk	0.1 g 1 g	2,700 10,800	215
3129	Glt-Ala-Ala-Pro-Leu-pNA	Pancreatic Elastase	Bulk	0.1 g 1 g	7,800 54,400	216
3066	Glu(pNA)	γ -Glutamyl Transpeptidase	Bulk	0.1 g 1 g 5 g	1,700 4,900 18,000	216
3067	Glu-pNA		Bulk	0.1 g 1 g	2,400 7,800	216
3074-v	Gly-Pro-pNA • Tos [GPNT]	X-Prolyl Dipeptidyl-Amino-peptidase	Vial	10 mg	2,700	218
3014	Leu-pNA	Aminopeptidase	Bulk	0.1 g 1 g 5 g	1,800 4,100 14,300	218
3130	Pyr-Phe-Leu-pNA	Thiol Protease	Bulk	0.1 g 1 g	4,800 29,700	224
(New) 3228-v	Pyr-Pro-Val-pNA	Human Granulocyte Elastase	Vial	5 mg	6,000	224
3071-v	Suc-Ala-Ala-Ala-pNA [STANA]	Elastase	Vial	5 mg	1,900	225
3071	Suc-Ala-Ala-Ala-pNA [STANA]		Bulk	0.1 g 1 g	4,400 26,000	
3117	Suc-Ala-Ala-pNA		Bulk	0.1 g 1 g	3,200 15,800	225
3162-v	Suc-Ala-Leu-Pro-Phe-pNA	PPase (Peptidyl Prolyl <i>cis-trans</i> Isomerase)	Vial	10 mg	10,000	225
3116	Suc-Ala-pNA		Bulk	0.1 g 1 g	2,500 8,800	225
3118	Suc-Ala-Pro-Ala-pNA	Elastase	Bulk	0.1 g 1 g	4,800 29,700	226
3127	Z-Ala-Ala-Leu-pNA	Subtilisin A, Serine Protease of <i>Bacillus subtilis</i> IFO3027, Neprilysin	Bulk	0.1 g 1 g	4,400 26,700	228
3111	Z-Gly-Gly-Leu-pNA	Neutral Endopeptidase	Bulk	25 mg 100 mg 1 g	5,200 14,000 91,000	229

Assay Method Using Peptidyl-*p*NA Substrates

Principle

A protease with limited specificity hydrolyzes a peptidyl-*p*NA substrate, releasing *p*-nitroaniline (*p*NA) as follows:



The initial rate of increase in the *p*NA concentration can be monitored photometrically at 405 nm.

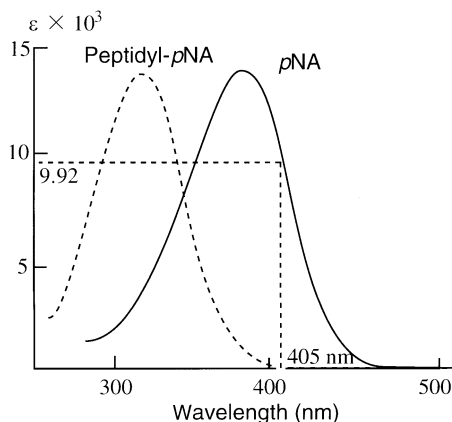


Fig. UV-Absorption Spectra

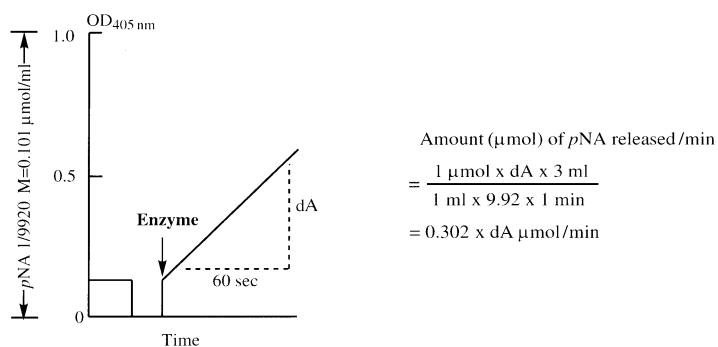
Reagents

- 1) Substrate stock solution: 10 mM (DMSO or distilled water)
- 2) Buffer
- 3) Enzyme solution

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the photometric method (initial-rate method).

- 1) Set a spectrophotometer at $\lambda = 405 \text{ nm}$ at 25°C ($\epsilon_{405 \text{ nm}} = 9920$)*
- 2) Pipette 2940 μl of buffer and 30 μl of substrate stock solution into the cuvette
- 3) Incubate in the spectrophotometer for 3-4 min (for temperature equilibration)
- 4) Add 30 μl of enzyme solution
- 5) Record the increase of the absorption intensity for 3-4 min
- 6) Calculate the amount of *p*NA released using the following equation



*R. Lottenberg, U. Christensen, C.M. Jackson, and P.L. Coleman, *In*, Proteolytic Enzymes Part C, *Methods in Enzymology*, **Vol. 80**, (L. Lorand, ed.) Academic Press, New York, 1981, pp. 341-361.

