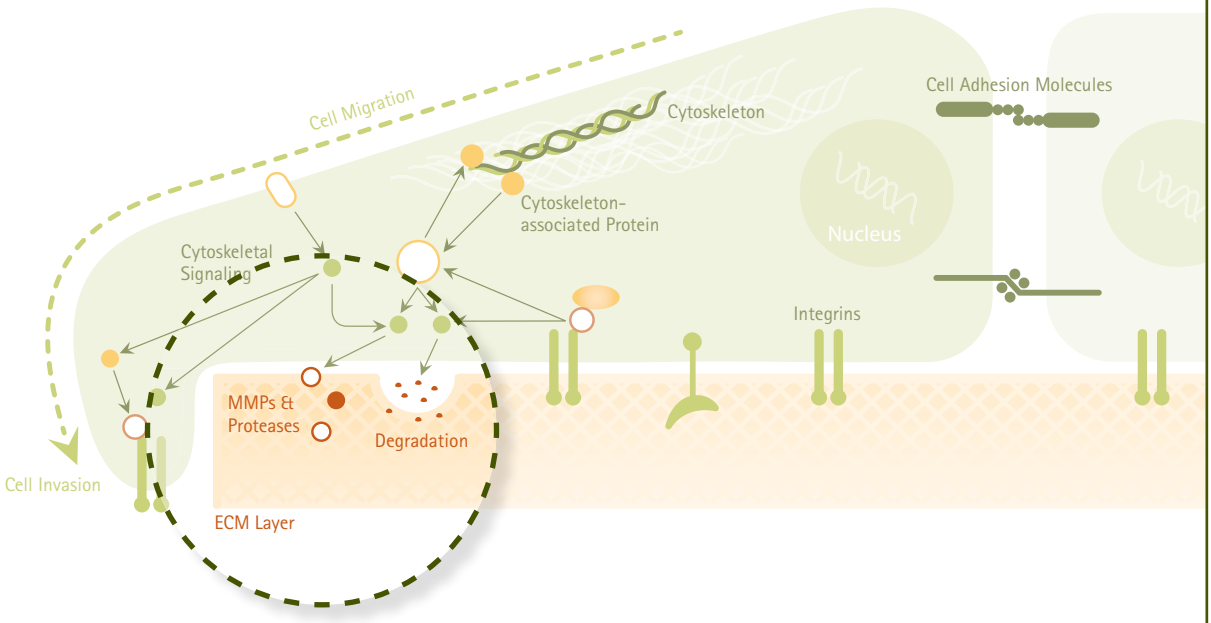
Matrix Metalloproteases: Activation for Activity Assays

Matrix metalloproteinases (MMPs) are secreted or transmembrane endopeptidases that process and degrade extracellular matrix proteins, such as collagen and elastin. Normal physiological roles for MMPs include neurite growth, tissue remodeling, wound healing, bone elongation and angiogenesis. Pathological processes involving MMPs include tumor growth, tumor cell migration and invasion, fibrosis and arthritis. MMPs are regulated in part by the tissue inhibitors of metalloproteinases (TIMPs) that bind MMPs non-covalently.

MMPs are also regulated by selective activation. Most MMPs are expressed as proenzymes, in which the catalytic amino acid(s) are blocked by the prodomain or by a chelated ion. Activation requires unblocking the catalytic residue, either by chemical induction or by proteolytic removal of the prodomain. Many proMMPs are activated by other MMPs or by other extracellular proteases. Non-proteolytic agents that activate MMPs include organomercurial chemicals (4-amino¬phenyl-mercuric acetate (APMA)), denaturants (SDS) and conformational perturbants (detergents).

Understanding MMP regulation, their physiological roles and specificity of action requires the generation of well-characterized, active preparations of MMPs. There are dozens of known MMPs and each has specific activating agents. To guide you in preparing activated forms of your MMPs of interest, the table below lists 15 MMPs, the conditions required for their activation, resulting cleavage products, relative activation level and associated literature references.



MMP Activation Table

Matrix Metalloproteinases (MMPs) are synthesized as latent pro-enzymes that require activation. The following table provides activating agents, termination conditions and relative activation levels designed as a resource & reference guide for activating various MMPs.

Enzyme	Synonyms	Latent Form MW_{appr} (kDa)	Activating Agent	Conditions	Activation Termination	Active Form MW_{appr} (kDa)			Mature Active Form N-terminal sequence	Relative Activation Level	Reference
						Intermediates	Mature Form	Calculated			
MMP-1	Collagenase-I, Fibroblast Collagenase, Intersitital Collagenase	57^{Gly} and 52 aa=51.8 acGly~1x5	APMA, 1mM	37˚C, 2-4h	A	44	43	42.5	V^{82}LTEG and L^{83}TEGN	1x	Grant et al., jbc 1987, 262, 5886; Fields et al. Biochemistry 1990, 29, 6671; Windsor et al., JBC 1994, 269,26201; Goldberg et al., JBC 1986, 261, 6600
			Trypsin^{TPCK}, 10 µg/mL	37˚C, 10-20 min	B	47					
			active-MMP-7 (1:1 molar ratio)	37˚C, slow	C		43	42.6	F^{81}VLTEG	low	Imai et al., jbc270/1995/6691; Reinemer et al., FEBS Lett. 1994/338/227
			APMA 1 mM + MMP-7 (1:1)	37˚C, 2-6h						6.5x	
			active-MMP-3	37˚C; slow			43	42.6	F^{81}VLTEG	low	Suzuki et al., Biochem 1990/29/10261, Nagase et al. Matrix Suppl. 1992 1:237, Murphy et al., Biochem J. 1987 248, 265
			APMA 1 mM + active-MMP-3	37˚C			$41^{C\text{-}term}$	40.8		5-8x	
MMP-2	Gelatinase A, 72kDa Gelatinase, Type IV Collagenase	72 aa=71.0	APMA, 1mM	37˚C, 1-2h	A		62	62.1	Y^{81}NFFPR	1x	Stetler-Stevenson et al., jbc 1989, 264, 1353; Nagase et al., Matrix Suppl. 1992 1:237 Crabbe FEBS Lett 1994 345 14, Okada Eur j biochem 1990 194 721
			Trypsin^{TPCK}, 10 µg/mL	does not activate							
			active-MMP-3	does not activate							
			active-MMP-7	37˚C, 8h	C				n.d.	0.6x	
MMP-3	Stromelysin-1	$(59\ \&\ 57)^{Gly}$ aa=52.2 acGly~1x5	APMA, 1 mM	37˚C, 6-12h	A	46	47^{Gly} and 45	42.8	F^{83}RTFPG	1x	Galazka bioch 1996 1996/34/11221; Nagase,bioch 1990/29/ 5783; Chen et al Biochem 1993/39/10289 Docherty, Murphy Ann Rheum.Dis 1990/49/469 Harrison, etal. Biochem 31,1992, 10757
			Trypsin^{TPCK}, 10 µg/mL	37˚C, 30 min	B	53	47^{Gly} and 45	42.8	F^{83}RTFPG	1x	
			active-MMP-7	does not activate							
MMP-7	Matrilysin, PUMP	28 aa=27.9	APMA, 1 mM	37˚C, 1h	A		19	19.1	Y^{78}SLFPNS	1x	Imai et al., JBC270/1995/ 6691 Crabbe, et.al. Biochem. 31,1992,8500
			Trypsin^{TPCK}, 10 µg/mL	37˚C, 1-2h	B						
			MMP-3 (5x molar excess)	37˚C, 8-16h	C						
MMP-8	Collagenase-2, Neutrophil collagenase	$(75\ \&\ 58^{N\text{-}trm})^{Gly}$ aa=51.1 ppGly~2x5 acGly~3x5	APMA, 1 mM	37˚C, 1-3h	A		58^{Gly}	41.9	M^{80}LTPGNP and L^{81}TPGNP	1x	Mallya Biochem 1990/29/10628; Tschesche Matrix Suppl. 1992 1/245
			Trypsin^{TPCK}, 10 µg/mL	37˚C, slow	B					Slow	
			MMP-3		C		58^{Gly}	41.9	F^{79}MLTPGNP	Superact.	Knauper, Biochem J. 1993/295/581; Reinemer, FEBS Lett. 1994/338/227; Farr et al Biochem. 1999; 38, 7332
MMP-9	92kDa Gelatinase, Gelatinase B	92Gly aa=76.3 ppGly~1x5 acGly~2x5	APMA, 1mM	37˚C, 16-24h	A	83	67	66.6*	M^{75}RTPR	1x	Wilhelm et al., 1989, Imai,JBC270/1995/6691; Okada, JBC267,1992, 21712; Shapiro JBC 270,1995,6351
			Trypsin^{TPCK}, 10 µg/mL	37˚C, 2h	B	74 and 68	64	66.6*	A^{74}MRTPR; C-term cleavage	1x	Tschesche et al.1992, Matrix Supp 1. 245, Okada, JBC267/1992/21712
			activeMMP-7 1:1	37˚C, 4h	C	83 & 80	78	74.6*	L^{16}RTNL; C-term cleavage	0.25x	Imai, jbc270/1995/6691
			APMA, 1 mM + activeMMP-7 (1:1)	37˚C, 12h		83	62	66.6*	M^{75}RTPR & F^{88}QTFE; C-term cleavage	0.7x	Imai, jbc270/1995/6691
			Active MMP-3 (1:1)	37˚C, 1-2h		82 (70)	67 (64)	66.6*	F^{88}QTFE; N-term & C-term cleavage	1x	Okada, JBC267/1992/21712; Shapiro JBC 270,1995,6351
MMP-10	Stromelysin-2		Like MMP-3								
MMP-11	Stromelysin-3	62	APMA	does not activate							Santavicca Biochem J 1996 315, 953
			Furin	intracellularly			47			1x	
MMP-12	Macrophage Metalloelastase	54	Autolytic	Refolding in Ca^{2+}/Zn^{2+} buffer		45	22		N-tem & C- term cleavage	1x	Shapiro et al., JBC 267/1992/ 4664
MMP-13	Collagenase-III	60	APMA, 1mM	37˚C, 30-60 min	A		48		Y^{85}NVFPRT	1x	Knauper et al., JBC 271/1996/1544
			MMP-3 (1/10 moles)	37˚C; 3-7h	C						
			Trypsin^{TPCK}, 10 µg/mL	37˚C: 10-30 min	B						
MMP-14	MT1-MMP	$31^{rProCatDom.}$	APMA	Does not activate			25 and $23^{C\text{-}term\ trunc}$				Will et al., JBC 271/1996/17119
			Furin	Intracellularly							
			Trypsin^{TPCK}, 5 µg/mL	37˚C; 10-60 min	B				YAIGGLKW	1x	
MMP-15	MT2-MMP	$33^{rProCatDom.}$	Autolytic for rPro	Refolding in Ca^{2+}/Zn^{2+} buffer		30	24 and 22		L84 & L93	1x	Kolkenbrock et al., Biol.Chem. 378/1997/ 71
MMP-16	MT3-MMP	rProCatDom	Autolytic for rPro	Refolding in Ca^{2+}/Zn^{2+} buffer			21		L91		Shofuda et al. JBC 272/1997/ 9749
MMP-17	MT4-MMP	rProCatDom	Trypsin^{TPCK}, 5 µg/mL	37˚C; 30-120 min		Pei and Weiss. JBC 271/ '96/ 9135	24				Takino et al., JBC 270/1995/ 23013
MMP-24	MT5-MMP	63	nd	Intracellularly		40-46	29				Pei JBC 274/1999/ 8925

Activation Buffer:
50mM Tris; 0.15M NaCl; 10mM$CaCl_2$; 0.05% Brij 35

Activation Termination:
A Spin column
B PMSF 2mM; Aprotinin 10 µg/mL; Soybean Trypsin Inhibitor 2-10x of the Trypsin amount
C EDTA 20mM; o-Phenantroline 1 mM (will inactivate the enzyme MMP and the activator MMP)

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