

# Proteases



Numerous members of the metalloprotease and cysteine, aspartic, and serine protease classes have been implicated in the etiology of various human diseases including Alzheimer's disease, cancer, and cardiovascular disease, among others. The metalloproteases (upper left), including the ACEs (angiotensin converting enzymes), ADAMs (a disintegrin and metalloprotease-like domain proteins), MMPs (matrix metalloprotease), and NEPs (nepilysins), all require a metal ion (often zinc) for catalysis and are implicated in such diverse functions as reproduction, development, tissue remodeling, and immunity. The cysteine proteases (lower right), including the caspases and most of the cathepsins, all require a critical cysteine residue for catalysis and are involved in apoptosis, inflammation, and protein degradation. The aspartic proteases (lower left) all require a particular aspartate residue for catalysis and are involved in angiogenesis, antigen processing, and protein activity regulation. The serine proteases (upper right), including the KLKs (kallikreins) and others, all require a specific serine residue for catalysis and play a role in many processes such as cancer.

# Proteases

## METALLOPROTEASES

*require a metal ion (zinc in most cases) for activity*

**ACEs** ACE and ACE-2, cell surface proteases, are important regulators of the renin-angiotensin system (RAS), which plays a key role in maintaining blood pressure homeostasis and fluid salt balance in mammals.<sup>1,2</sup> Because of its location and specificity, ACE plays additional roles in immunity, reproduction and neuropeptide regulation. ACE exists in two isoforms. Somatic ACE comprises two highly similar protease domains, whereas germinal ACE includes a single protease domain. Soluble ACE is derived from the membrane, forms by actions of secretases or sheddases, and is present in many biological fluids. ACE-2 consists of a single protease domain and is a negative regulator of RAS in the heart.

**ADAMs** There are over 30 membrane proteins in this family, about half of which are active metalloproteases including ADAM8, 9, 10, 12, 15 and 17/TACE.<sup>3,5</sup> They share a common domain structure including pro, metalloprotease, disintegrin-like, cysteine-rich, transmembrane, and cytoplasmic domains. They function in many important processes, such as fertilization and development, through their activities in cell adhesion/fusion, membrane protein shedding, and signal transduction. ADAMTSs (18 are known) have a similar domain structure except that they contain thrombospondin type 1 motifs.<sup>6</sup> ADAMTSs are extracellular proteases, cleaving targets such as aggrecan (ADAMTS1, 4 and 5), procollagens (ADAMTS2, 3 and 14) and von Willebrand factor (ADAMTS13).

**MMPs** There are 23 known human members in this family.<sup>7</sup> With a few exceptions, MMPs have a common domain structure including pro, metalloprotease, and hemopexin-like domains. MMP-7 and MMP-26 contain the pro and protease domains only, whereas MT-MMPs contain a C-terminal membrane-anchoring domain. MMPs play an important role in many physiological and pathological processes, such as embryonic development, morphogenesis, reproduction, tissue remodeling, arthritis, cancer, and cardiovascular disease. They function in the breakdown of extracellular matrix and in the processing of a variety of biological molecules. MMP activities are regulated at multiple levels, such as the activation of proenzymes and the inhibition of active enzymes by TIMPs (tissue inhibitor of metalloproteases; TIMP-1, -2, -3, and -4).

**NEPs** There are 8 known cell surface proteins in this family.<sup>8</sup> Nephilysin (NEP) is involved in the degradation of brain enkephalins and the inactivation of circulating atrial natriuretic peptide. It is also known as the common acute lymphoblastic leukemia antigen (CALLA) and serves as a major degrading enzyme of amyloid  $\beta$  peptide (A $\beta$ ) in the brain. Therefore, the inactivation or activation of NEP may be beneficial depending upon the tissues and diseases involved. Other NEP family members include ECEs (endothelin-converting enzymes), KELL (an erythrocyte cell-surface antigen), PEX (phosphate regulating gene with homologies to endopeptidases on the X chromosome), XCE (X-converting enzyme) or ECE-like 1, DINE (damage-induced neuronal endopeptidase), and SEP (secreted endopeptidase).

## CYSTEINE PROTEASES

*require a cysteine residue for activity*

**Caspases** These cytosolic aspartate-specific cysteine proteases are involved in the activation of certain pro-inflammatory cytokines and the initiation and execution of apoptosis.<sup>9,10</sup> They are expressed as latent zymogens and are activated by an autoproteolytic mechanism or via processing by other proteases (frequently other activated caspases) in response to an initiating signaling event. The human caspases can be subdivided into three functional groups: cytokine activation (caspase-1, -4, -5, and -13), apoptosis initiation (caspase-2, -8, -9, and -10) or apoptosis execution (caspase-3, -6, and -7). Caspase activation and activity can be negatively regulated by members of the inhibitor of apoptosis (IAP) gene family, including NAIP, XIAP, cIAP-1, cIAP-2, Livin, and Survivin. SMAC/Diablo and HtrA2/Omi, released from mitochondria during the initiation of apoptosis, can reverse the inhibitory effects of IAPs on caspases.

**Cathepsins** There are 11 human members (cathepsins B, C, F, H, K, L, O, S, V, W and X) and 8 additional mouse members (cathepsins 1, 2, 3, 6, J, M, Q and R) in this group of the papain protease family.<sup>11,12</sup> As lysosomal proteases, cathepsins play an important role in protein degradation. Due to their broad substrate specificity, cathepsins are capable of activating or inactivating a wide range of molecules. As evidenced by redistribution or elevation in human and animal tumors, several cathepsins such as B, H, and L may participate in tumor cell invasion and metastasis. Expression and activity of cathepsins are regulated at several levels. For example, cathepsins are synthesized as inactive proenzymes and processed to become mature and active enzymes. Endogenous protein inhibitors, such as cystatins and serpins, inhibit active enzymes.<sup>13,14</sup>

# Proteases *continued*

## ASPARTIC PROTEASES

*require an aspartate residue for activity*

**Pepsins** BACE ( $\beta$ -site amyloid precursor protein cleaning enzyme) -1 and -2 and cathepsins D and E are members of the pepsin protease family. All are candidates for the  $\beta$ -secretase activity that generates A $\beta$  peptide from the amyloid precursor protein (APP).<sup>15</sup> Among them, BACE-1 has gained the most interest as a drug target because it is the principal  $\beta$ -secretase in neurons and BACE-1 null mice are viable.<sup>16</sup> BACEs are type I membrane proteins with two active site motifs. BACE-1 is expressed in a variety of human tissues and it is likely that this peptidase has additional functions. As a lysosomal protease, cathepsin D is a major contributor to protein degradation. As a secreted protease from prostate carcinoma cells, cathepsin D is responsible for the generation of angiostatin, a potent endogenous inhibitor of angiogenesis.<sup>17</sup> Cathepsin E is not a lysosomal protease and has a limited cell and tissue distribution. Both cathepsins play important roles in the generation of bioactive proteins and in antigen processing.<sup>17</sup>

## SERINE PROTEASES

*require a serine residue for activity*

**Kallikreins** There are at least 15 known human genes belonging to this serine protease family, all of which map to the same chromosomal locus and are regulated by steroid hormones. The kallikreins (KLKs) are implicated in various human diseases.<sup>18</sup> For example, KLK3 encodes prostate-specific antigen (PSA), a marker for prostate cancer, and KLK5 is overexpressed in ovarian carcinomas.

**Additional serine proteases** Cathepsin A is a member of the serine carboxypeptidase family.<sup>19</sup> DPP/IV/CD26 is a member of the serine prolyl protease family.<sup>20</sup> HGF activator (HGFA) is a serine endopeptidase that cleaves the single-chain human HGF precursor, generating the active heterodimer. HGFA can be activated by autocatalysis or by thrombin, a key serine protease in the clotting pathway.<sup>21</sup> Active HGFA can be inhibited by HGFA inhibitors (HAIs). Two HAIs, HAI-1 and HAI-2, have been identified in mouse and human. HAI-1 is not only an inhibitor, but also a specific acceptor of active HGFA, acting as a reservoir of this enzyme on the cell surface.<sup>22</sup>

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# PROTEASE ALIASES

### ADAM Family Aliases

- 8 - CD156a, MS2
- 9 - MDC9, Meltrin- $\gamma$ , MCMP
- 10 - MADM
- 12 - Meltrin- $\alpha$
- 15 - MDC15, Metargidin
- 17 - TACE
- TS4 - Aggrecanase 1, ADMP-1
- TS5 - Aggrecanase 2, ADMP-2

### Caspase Family Aliases

- 1 - ICE
- 2 - NEDD2, ICH-1
- 3 - CPP32, YAMA, Apopain, LICE-1
- 6 - Mch2
- 7 - Mch3, ICE-LAP3, CMH-1, LICE-2
- 8 - Mch5, MACH, FLICE-1
- 9 - Mch6, ICE-LAP6, APAF-3
- 10 - Mch4, FLICE-2

### Cathepsin Family Aliases

- A - Lysosomal Carboxypeptidase A
- C - DPP1
- D - Lysosomal Aspartyl Protease
- L - MEP

### MMP Family Aliases

- 1 - Interstitial Collagenase, Collagenase-1
- 2 - Gelatinase A, 72-kDa Gelatinase
- 3 - Stromelysin-1, Transin
- 7 - Matrilysin, PUMP
- 8 - Collagenase-2, Neutrophil Collagenase
- 9 - Gelatinase B, 92-kDa Gelatinase
- 10 - Stromelysin-2
- 11 - Stromelysin-3
- 12 - Macrophage Elastase
- 13 - Collagenase-3
- 14 - MT1-MMP
- 15 - MT2-MMP
- 24 - MT5 - MMP



*For more protease aliases or for aliases to cytokines and related factors, please use the reply card to request a copy of R&D Systems' aka CD-ROM.*

*For a poster on MMPs and Cytokines (including cytokine substrates and amino acid sequences cleaved, cytokine inducers and inhibitors of MMP expression, and references), please use the reply card to request a copy.*

# Protease Products from R&D Systems

Name	Recombinant Proteins	Polyclonal Antibodies	Monoclonal Antibodies	Assay Kits	ELISA Development Kits	Primer Pairs
<b>METALLOPROTEASES</b>						
ACE	●					
ACE-2	●					
ADAM8		●	●	●		●
ADAM9	● ■	● ■	● ■			
ADAM10	● ■	■	■			
ADAM12						●
ADAM15		● ●	●			●
ADAM17/TACE	●	●	●			●
ADAMTS4						●
ADAMTS5						●
MMP-1	●	●	●	●		●
MMP-2	● ■	●	●	●		● ■
MMP-3	● ■	● ■	● ■	●		●
MMP-7	●	●	●	●		●
MMP-8	●	●	●	●		●
MMP-9	● ■	● ■	● ■	● ■		●
MMP-10	●	●	●	●		
MMP-11						●
MMP-12	●		●	●		
MMP-13	●	●	●	●		
MMP-14			●			
MMP-15			●			
MMP-24		● ■	■			
Neprilysin	● ■					● ■ ▲
<b>CYSTEINE</b>						
Caspase-1/ICE				● ●		
Caspase-2	●	● ■		● ●		
Caspase-3	●	● ■ ◆	●	● ●		
Caspase-6				● ●		
Caspase-7	●	● ■		●		
Caspase-8	●	●	●	●		
Caspase-9		●		● ●	●	
Caspase-10	●	●	●	●		
Cathepsin B	● ■	●		●		
Cathepsin C/D/PP1	● ■					
Cathepsin H	■	■				
Cathepsin L	●	●				
Cathepsin O		●				
Cathepsin S	●					
Cathepsin V	●					
Cathepsin X/Z/P	● ■	● ■				
<b>ASPARTIC</b>						
BACE-1	●	●	●			● ■
Cathepsin D	● ■	● ■				
Cathepsin E	■					
<b>SERINE</b>						
Cathepsin A	● ■					
DPP1V/CD26	● ■	■	■			
HGFA	■					
Kallikrein 5	●					
Thrombin		●				

Protease inhibitors and substrates are also available. For more information, please refer to our website at [www.RnDSystems.com](http://www.RnDSystems.com)

**Key to product table** ● Human ■ Mouse ▲ Rat ◆ Multi-species

All products are for research use only. Not for use in humans. Not for use in diagnostic or therapeutic procedures.

# Recent References

## ACTIVITY ASSAYS

**Caspase-1 (Catalog # BF12100)**

**Caspase-2 (Catalog # BF5100)**

**Caspase-3 (Catalog # BF1100)**

**Caspase-6 (Catalog # BF6100)**

**Caspase-8 (Catalog # BF2100)**

**Caspase-9 (Catalog # BF7100)**

Yang, S.-E. *et al.* (2002) *Biochem. Pharmacol.*

**63:1641.** "Down-modulation of Bcl-X<sub>L</sub> release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells."

**MMP-9 (Catalog # DMP900)**

Galley, H.F. *et al.* (2002) *Anaesthesia* **57:659.**

"Matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and tumor necrosis factor  $\alpha$  release during cardiopulmonary bypass."

## ANTIBODIES

**Caspase-3 (Catalog # AF835)**

Kim, D.-H. *et al.* (2002) *Ophthalmic Res.*

**34:150.** "Activation of caspase-3 during degeneration of the outer nuclear layer in the rd mouse retina."

**MMP-9 (Catalog # AF909)**

Huang, S. *et al.* (2002) *J. Natl. Cancer Inst.*

**94:1134.** "Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice."

## PROTEINS

**Caspase-3 (Catalog # 707-C3)**

Greidering, E.L. *et al.* (2002) *Arthritis Rheum.* **46:1264.** "Apoptotic U1-70 kd is antigenically distinct from the intact form of the U1-70-kd molecule."

**MMP-9 (Catalog # 911-MP)**

Robinson, S.C. *et al.* (2002) *Eur. J. Immunol.*

**32:404.** "Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNF- $\alpha$ ."

# Secretase Activity Assays

Amyloid beta ( $A\beta$ ) peptide is formed as a result of amyloid precursor protein (APP) cleavage via  $\beta$ -secretase and  $\gamma$ -secretase ( $\alpha$ -secretase activity has also been described). The cleavage domains of APP targeted by these enzymes are membrane proximal, suggesting that secretases may also be membrane bound proteins. ADAM17/TACE and ADAM10 exhibit  $\alpha$ -secretase activity,<sup>1</sup> while  $\beta$ -secretase activity has been attributed to the peptidatin-sensitive enzyme BACE.<sup>2,3</sup> Although the identity of  $\gamma$ -secretase remains elusive, a number of candidates have been proposed, including presenilin-1 and presenilin-2 (Table 1).<sup>4</sup>

In Figures 1-3, the substrate specificities of the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretases are shown. Cleavage activity associated with each of these three secretases was monitored using caged fluorescent reporter (EDANS-DABCYL) substrates that encompass the three distinct secretase cleavage sites of the APP protein. Recombinant enzymes were the source of  $\alpha$ - and  $\beta$ -secretase activities, while mouse brain, a tissue rich in secretase activity, was the source of the  $\gamma$ -secretase activity. The lack of cross-reactivity in enzymatic action for each of the different substrate sequences selected made it possible to follow the three distinct secretase activities.

ACTIVITY	CANDIDATES
$\alpha$ -Secretase	ADAM10, <sup>5</sup> ADAM17, <sup>5</sup> Prohormone convertase 7, <sup>5</sup> BACE-2 <sup>6</sup>
$\beta$ -Secretase	BACE-1, <sup>2</sup> BACE-2, <sup>2</sup> Cathepsin D, <sup>7</sup> Cathepsin E <sup>7</sup>
$\gamma$ -Secretase	Presenilin-1, <sup>8</sup> Presenilin-2, <sup>8</sup> Nicastrin, <sup>9</sup> Herp, <sup>10</sup> APh-1, <sup>11</sup> PEN-2 <sup>11</sup>
Degradation	Nephrilysin, <sup>12</sup> Endothelin-converting enzyme, <sup>13</sup> Insulin-degrading enzyme <sup>13</sup>

Table 1. Protein candidates involved in  $A\beta$  metabolism.

## References

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## AVAILABLE KITS

*These kits are applicable for monitoring or comparing specific secretase activities between different tissue sources as well as in identifying possible inhibitors of these enzymes. They may also be useful in determining the role of secretases in Notch processing and signaling. While kit substrates are specific for  $\alpha$ -,  $\beta$ -, or  $\gamma$ -Secretases, they may also be cleaved by other proteases endogenous to the tissue sample tested.*

KIT	CATALOG #	SIZE
$\alpha$ -Secretase	FP001	1 Kit
$\beta$ -Secretase	FP002	1 Kit
$\gamma$ -Secretase	FP003	1 Kit

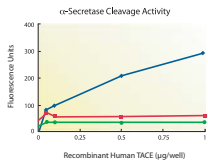


Figure 1. Recombinant TACE exclusively cleaves the  $\alpha$ -secretase substrate (blue) and is unable to cleave the  $\beta$ - and  $\gamma$ -substrate (red and green, respectively).

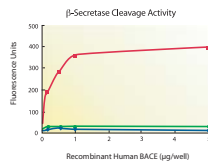


Figure 2. Recombinant BACE exclusively cleaves the  $\beta$ -secretase substrate (red), demonstrating no activity on either the  $\alpha$ - or  $\gamma$ -substrate (blue and green, respectively).

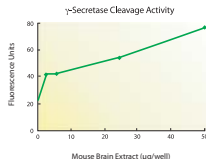


Figure 3. Exposure to mouse brain extract (i.e. a tissue known to contain  $\gamma$ -secretase activity) results in cleavage activity for the  $\gamma$ -substrate sequence (green).

For a list of references citing the use of R&D Systems' assays, please download a copy of the latest ELISA Reference Guide & Catalog at [www.RndSystems.com/media/ERG2003.pdf](http://www.RndSystems.com/media/ERG2003.pdf)

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Minneapolis, MN 55413, USA  
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R&D Systems Europe Ltd.  
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK  
Tel: +44 (0)1235 551100  
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R&D Systems GmbH  
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reagent, however, allows for activation of the total sample and is included in each Fluorokine E kit. Both assays are complete kits and are fully validated for the sample types indicated in the protocol manual.

## AVAILABLE KITS

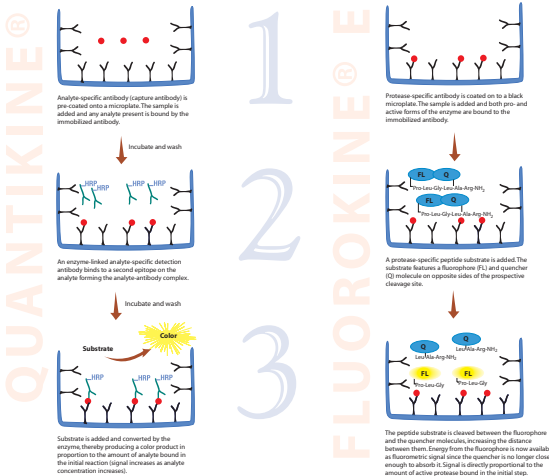
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MMP-2	Human	DMP200
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MMP-7	Human	DMP700
MMP-8	Human	DMP800
MMP-9	Human	DMP900
Pro-MMP-9	Mouse	MMP900
MMP-10	Human	DM1000
Pro-MMP-13	Human	DM1300
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TIMP-2	Human	DTM200

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Active MMP-9	Human	F9M00
Active MMP-13	Human	F13M00



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