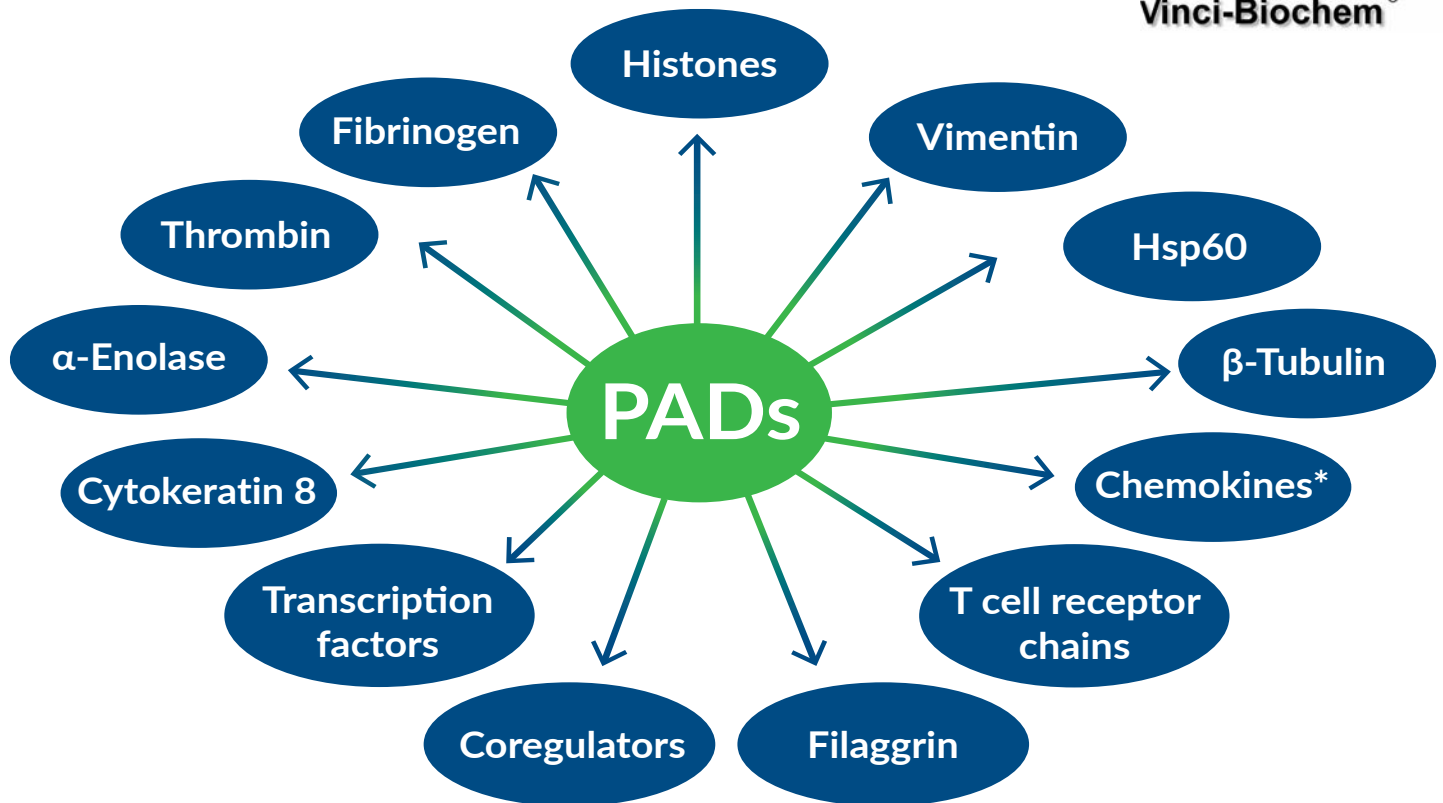


# Citrullination Research Tools

Citrullination is a post-translational modification in which positively charged arginine is deiminated to a neutrally charged citrulline. Protein arginine deiminases (PADs) catalyze this process, which ultimately alters a protein's function. Citrullination has normal roles in gene regulation as well as pathological roles. Of the known PAD isotypes, PAD2 and PAD4 are the most studied due to their impact on inflammation, epigenetics, cancer, and autoimmune diseases. Known substrates that are targeted by PADs are listed in the graphic below.



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Citrullination Targets

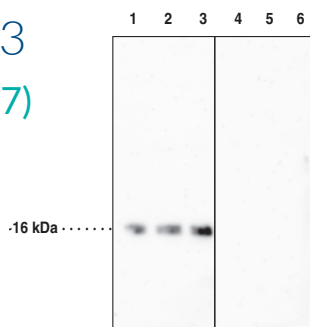
\*CXCL5, CXCL8/IL-8, CCL17, CCL26

## Proteins and Purified Enzymes

Item No.	Product Name	Description
20582	Citrullinated Core Histones (bovine)	A mixture of H1, H2A, H2B, H3, and H4 histones isolated from calf thymus and modified by PAD4
17926	Citrullinated Histone H3 (human recombinant)	Purified protein expressed in <i>E. coli</i>
18473	Human Fibrinogen (PAD2 Citrullinated)	Native protein purified from human plasma and citrullinated with human recombinant PAD2
400076	Human Fibrinogen (PAD4 Citrullinated)	Native protein purified from human plasma and citrullinated with human recombinant PAD4
10784	PAD1 (human recombinant)	Active, N-terminal His-tagged enzyme expressed in <i>E. coli</i>
10785	PAD2 (human recombinant)	Active, N-terminal His-tagged enzyme expressed in insect cells
10786	PAD3 (human recombinant)	Active, N-terminal His-tagged enzyme expressed in <i>E. coli</i>
10500	PAD4 (human recombinant)	Active, N-terminal His-tagged enzyme expressed in <i>E. coli</i>
13188	Thrombin (human)	Sourced from human blood

# Detect Citrullinated Human H3 Histone H3 (Citrullinated R2 + R8 + R17) Monoclonal Antibody - Item No. 17939

- Species Reactivity: Human
- Applications: ELISA, WB



Lane 1: Citrullinated Human H3 (10 ng)  
 Lane 2: Citrullinated Human H3 (25 ng)  
 Lane 3: Citrullinated Human H3 (50 ng)  
 Lane 4: Histone H3 (human recombinant) (Item No. 10263) (10 ng)  
 Lane 5: Histone H3 (human recombinant) (Item No. 10263) (25 ng)  
 Lane 6: Histone H3 (human recombinant) (Item No. 10263) (50 ng)

## Antibodies to PADs and Their Common Substrates

Item No.	Product Name	Species Reactivity	Application(s)
23000	Citrullinated $\alpha$ -Enolase Monoclonal Antibody (Clone 8D3)	Human	ELISA, WB
17088	Citrullinated Fibrinogen Monoclonal Antibody (Clone 10E9.3)	Human	ELISA, WB
22054	Citrullinated Vimentin Monoclonal Antibody (Clone 12G11)	Human	ELISA, IP, WB
10004600	Cytokeratin Monoclonal Antibody (Clone C-11)	Human	FC, IF, IHC, WB
10349	Cytokeratin Monoclonal FITC Antibody (Clone C-11)	Human	FC, IF
10478	Cytokeratin Monoclonal PE Antibody (Clone C-11)	Human	FC, IF
20491	$\alpha$ -Enolase Polyclonal Antibody	Human	ELISA, WB
18793	Fibrinogen ( $\alpha$ chain) Monoclonal Antibody (Clone 6D6)	Human	WB
18033	Fibrinogen ( $\alpha$ chain) Polyclonal Antibody	Human	WB
18073	Histone H1.4 (Citrullinated R53) Polyclonal Antibody	Human	WB
13781	Histone H3 Polyclonal Antibody	Human	ELISA, FC, IP, WB
17855	Histone H3 (Citrullinated R2 + R8 + R17) Polyclonal Antibody	Human	ELISA, WB
10011429	Hsp60 Monoclonal Antibody (Clone LK-1)	Human, multiple animal species	ELISA, FC, IP, WB
10011430	Hsp60 Monoclonal Antibody (Clone LK-2)	Human, multiple animal species	FC, IHC, WB
22997	PAD1 Monoclonal Antibody (Clone 6B4)	Human	ELISA, WB
19822	PAD2 Monoclonal Antibody (Clone 9F7)	Human	ELISA, WB
19669	PAD4 Monoclonal Antibody (Clone 6D8)	Human	ELISA, WB
19671	PAD4 Monoclonal Antibody (Clone 11F9)	Human	ELISA, WB
20197	Vimentin Monoclonal Antibody (Clone 12E4)	Human	ELISA, WB

## Reagents for Studying Citrullinated Proteins

Item No.	Product Name	Description
17079	BB-CI-Amidine	Potent, stable pan-PAD inhibitor with increased cellular potency ( $EC_{50}$ = 8.8 $\mu$ M in cells for PAD4)
10599	CI-Amidine (trifluoroacetate salt)*	Irreversible inhibitor of PAD1, PAD3, and PAD4 ( $IC_{50}$ s = 0.8, 6.2, and 5.9 $\mu$ M, respectively)
10610	F-Amidine (trifluoroacetate salt)*	Irreversible inhibitor of PAD1, PAD3, and PAD4 ( $IC_{50}$ s = 29.5, 350, and 21.6 $\mu$ M, respectively)
19933	AP-III-a4	Inhibits enolase ( $IC_{50}$ = 0.576 $\mu$ M); inhibits cancer cell migration and invasion processes
16172	Citrulline-specific Probe	Fluorescent probe for citrulline-containing protein detection
17489	GSK199 (hydrochloride)	Selective inhibitor of PAD4 ( $IC_{50}$ = 200 nM)
17488	GSK484 (hydrochloride)	Selective inhibitor of PAD4 ( $IC_{50}$ = 50 nM)
401272	Human Fibrinogen Affinity Sorbent	Immobilized human fibrinogen for the removal of antibodies that bind unmodified fibrinogen
11352	Withaferin A	Targets metastatic cells that upregulate vimentin expression

\*Sold under license from the University of South Carolina under U.S. Patent No. 7,964,636

## Assays for Studying PADs and Their Substrates

Item No.	Product Name	Description
501270	Anti-Citrullinated Human Fibrinogen Assay Kit (mouse)	Measure antibodies specific for citrullinated human fibrinogen in mouse plasma or serum
501620	Citrullinated Histone H3 (Clone 11D3) ELISA Kit	Detect CitH3 in cell lysates, cell culture supernatant, plasma, and serum
501450	PAD2 (human) ELISA Kit	Detect human PAD2 in cell culture supernatants, cell lysates, plasma, and serum
500930	PAD4 Autoantibody ELISA Kit	Measure anti-PAD4 autoantibodies of any isotype (IgM, IgG, IgA)
501460	PAD4 (human) ELISA Kit	Detect human PAD4 in tissue culture medium, cell lysates, plasma, and serum

### Screening Assays for Identifying Inhibitors of PAD Activity

Cayman has developed a set of orthogonal assays to enable the screening of large libraries of compounds for next generation PAD inhibitors. These fluorescence-based assays monitor the activity of either PAD2 or PAD4 by one of two methods: 1) detecting the ammonia released by the deimination reaction or 2) exploiting the substrate specificity of trypsin to monitor the citrullination reaction.

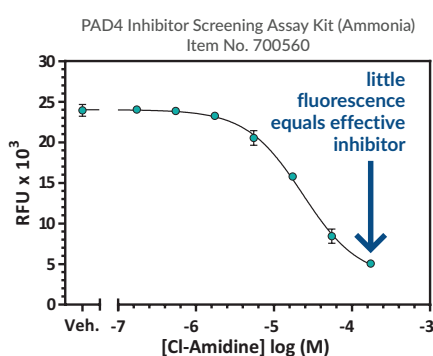
Item No.	Product Name	Readout
701390	PAD2 Inhibitor Screening Assay Kit (AMC)	Fluorescence plate reader (ex 355-365 nm, em 445-455 nm)
701400	PAD2 Inhibitor Screening Assay Kit (Ammonia)	Fluorescence plate reader (ex 405-415 nm, em 470-480 nm)
701320	PAD4 Inhibitor Screening Assay Kit (AMC)	Fluorescence plate reader (ex 355-365 nm, em 445-455 nm)
700560	PAD4 Inhibitor Screening Assay Kit (Ammonia)	Fluorescence plate reader (ex 405-415 nm, em 470-480 nm)

## Which PAD Inhibitor Screening Assay is Right for You?

### Ammonia Detection

Links citrullination to the amount of ammonia produced when a PAD enzyme deiminates a synthetic PAD substrate:

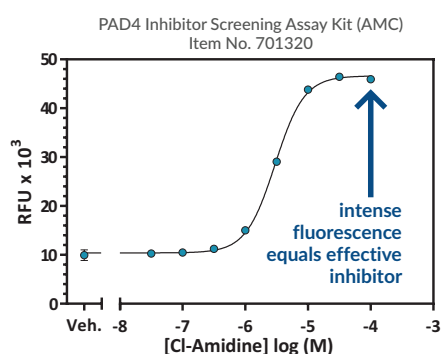
- Ammonia reacts with a detector, resulting in a fluorescent product.
- The fluorescent signal is reduced in the presence of an effective PAD inhibitor.



### AMC-tagged Arginine as a Trypsin Substrate

Links citrullination to the inability of trypsin to hydrolyze a modified PAD substrate:

- In the presence of an effective PAD inhibitor, the substrate will not be citrullinated, and free AMC is released with the addition of trypsin.
- The intensity of AMC fluorescence confirms inhibition of PAD activity and is less prone to false positives.



Discover our pre-clinical screening funnel to identify selective PAD4 inhibitors, available for download at [www.caymanchem.com/screeningcitrullination](http://www.caymanchem.com/screeningcitrullination)

# Related Products

## Tools to Investigate NETosis

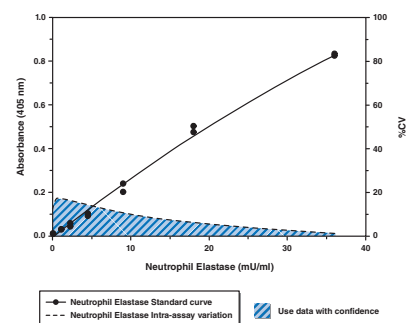
PADs have been linked to inflammation through their role in catalyzing histone H3 hypercitrullination during neutrophil extracellular trap (NET) formation. Cayman has developed a collection of tools to study this form of pathogen-induced cell death.

Item No.	Product Name	Description
11189	N-acetyl-Pro-Gly-Pro Peptide	A neutrophil chemoattractant
501410	Myeloperoxidase (human) ELISA Kit	Detect human MPO in cell culture supernatants, plasma, and serum
14922	Neutrophil Elastase Inhibitor	Selectively targets the binding domain of neutrophil elastase ( $IC_{50} = 7$ nM)
601070	Neutrophil (mouse) Isolation Kit	Isolate mouse neutrophils from peritoneal lavage or bone marrow

## Measure NET-Derived Neutrophil Elastase

### NETosis Assay Kit - Item No. 601010

- Induce and detect NET formation *in vitro*
- Elastase-based readout
- Non-dsDNA readout eliminates false positives from DNA
- Adaptable to multiple species



## Carbamylated Antibody and Proteins

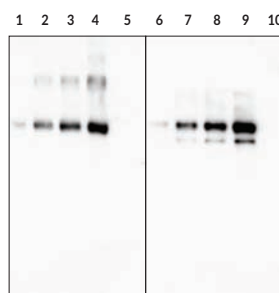
Carbamylation is a post-translational modification that results in the generation of homocitrulline *via* the non-enzymatic reaction of cyanate with lysine. Cayman developed a polyclonal antibody to detect carbamylated proteins and recombinant versions of key carbamylated proteins.

Item No.	Product Name	Description
21075	Carbamylated Bovine Serum Albumin	Albumin isolated from bovine plasma and modified with potassium cyanate
21076	Carbamylated Core Histones (bovine)	A carbamylated mixture of H1, H2A, H2B, H3, and H4 histones isolated from calf thymus
21097	Carbamylated Fetal Calf Serum	Fetal calf serum modified with potassium cyanate
21370	Carbamylated Human Fibrinogen	Fibrinogen purified from human plasma and modified with potassium cyanate

## Detect Carbamylated Proteins

### Anti-Carbamylation (Homocitrulline) Polyclonal Antibody - Item No. 22428

Specifically detects carbamylated proteins of any type and from any species



Western blot using the Anti-Carbamylated (Homocitrulline) Polyclonal Antibody.

Lane 1: Carbamylated BSA (1 ng)  
 Lane 2: Carbamylated BSA (5 ng)  
 Lane 3: Carbamylated BSA (10 ng)  
 Lane 4: Carbamylated BSA (25 ng)  
 Lane 5: BSA (300 ng)  
 Lane 6: Carbamylated Fibrinogen (1 ng)  
 Lane 7: Carbamylated Fibrinogen (5 ng)  
 Lane 8: Carbamylated Fibrinogen (10 ng)  
 Lane 9: Carbamylated Fibrinogen (25 ng)  
 Lane 10: Fibrinogen (300 ng)



To view a complete list of our citrullination research tools, visit us online at [www.caymanchem.com](http://www.caymanchem.com)

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