PRODUCT INFORMATION



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

Overview and Properties

Contents: Synonym: Immunogen:	This vial contains 500 μl of hapten affinity-purified IgG. pan-Carbamylation Carbamylated protein
Cross Reactivity:	(+) Carbamylated proteins (-) Citrullinated proteins
Species Reactivity:	(+) Human carbamylation (homocitrulline)
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly
Storage Buffer:	TBS, pH 7.4, containing 50% glycerol, 0.1% BSA, and 0.02% sodium azide
Host:	Rabbit
Applications:	ELISA, Immunoprecipitation (IP), and Western blot (WB); the recommended starting dilution for ELISA and WB is 1:1,000 and 2-5 μ g of antibody per IP test. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

Images



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





Western blot against Carbamylated HeLa cell lysates.

Lane 1:	HeLa Cell Lysates (5 µg)
Lane 2:	HeLa Cell Lysates (10 µg)
Lane 3:	Citrullinated HeLa Cell Lysates (5 µg)

Lane 4:	Citrullinated HeLa Cell Lysates (10 µg)
Lane 5:	Carbamylated HeLa Cell Lysates (5 µg)
Lane 6:	Carbamylated HeLa Cell Lysates (10 µg)
Lane 7:	Carbamylated BSA (5 ng)
Lane 8:	Carbamylated BSA (25 ng)
Lane 9:	Carbamylated Fibrinogen (5 ng)

Lane 10: Carbamylated Fibrinogen (25 ng)



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

Lane 1: Citrullinated Histone H3 (25 ng) Lane 2: Citrullinated Histone H3 (100 ng) Lane 3: Histone H3 (200 ng) Lane 4: Citrullinated Fibrinogen (25 ng) Lane 5: Citrullinated Fibrinogen (100 ng) Lane 6: Fibrinogen (200 ng) Lane 7: Citrullinated Alpha Enolase (25 ng) Lane 8: Citrullinated Alpha Enolase (210 ng) Lane 9: Alpha Enolase (200 ng)



Immunoprecipitation (IP) with the Anti-Carbamylation (Homocitrulline) Polyclonal Antibody. Antibodies were coupled to Protein A resin [5] by of antibodies coupled to 25 µl of resin) and incubated with the indicated proteins. The IP reactions were eluted and run on SDS-PAGE, followed by Western blotting using Fibrinogen (a chain) Monoclonal Antibody (Clone 6D6) (Item No. 18793).

Monocinal Antibody: Carbamylated Elbrinogen (2 µl IP reaction) Lane 2: No Antibody: Carbamylated Elbrinogen (2 µl IP reaction) Lane 3: Nogative Control Polyclonal Antibody: Carbamylated Elbrinogen (2 µl IP reaction) Lane 4: Negative Control Polyclonal Antibody: Carbamylated Elbrinogen (2 µl IP reaction) Lane 5: Anti-Carbamylation Polyclonal Antibody: Carbamylated Fibrinogen (2 µl IP reaction) Lane 6: Anti-Carbamylation Polyclonal Antibody: Carbamylated Fibrinogen (2 µl IP reaction) Lane 7: Anti-Carbamylation Polyclonal Antibody: Carbamylated Fibrinogen (2 µl IP reaction) Lane 7: Anti-Carbamylation Polyclonal Antibody: Carbamylated Fibrinogen (2 µl IP reaction) Lane 7: Anti-Carbamylation Polyclonal Antibody: Carbamylated Fibrinogen (2 µl IP reaction) Lane 9: Fibrinogen (1 + Wester blot control) (50 ng)

WARNING THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFFTY DATA

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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PRODUCT INFORMATION



Description

Cayman's Anti-Carbamylation (Homocitrulline) Polyclonal Antibody specifically detects carbamylated proteins and does not detect the unmodified or citrullinated counterparts.

Carbamylation is the non-enzymatic and irreversible post-translational modification whereby cyanate reacts with lysine residues within polypeptide chains to generate ε-carbamyl-lysine (homocitrulline).¹ Cyanate originates from the decomposition of urea and exists in equilibrium with its reactive form isocyanic acid.² Neutrophil derived MPO mediates the conversion of isocyanate from thiocyanate at sites of inflammation.^{1,3} Increased levels of urea associated with chronic kidney disease result in elevated cyanate concentrations, and a higher potential for carbamylated proteins.⁴ The presence of carbamylated proteins has been associated with rheumatoid arthritis.⁵ Homocitrulline residues are structurally similar to citrulline; the presence of an additional methylene group on homocitrulline being the only difference. Peptidyl arginine deiminase (PAD) mediated citrulline residues on proteins and peptides can indicate many of the same disease states as the presence of homocitrulline residues. The ability to differentiate between citrullinated and carbamylated proteins has been difficult using traditional techniques.

References

- 1. Verbrugge, F.H., Tang, W.H.W., and Hazen, S.L. Protein carbamylation and cardiovascular disease. *Kidney Int.* **88(3)**, 474-478 (2015).
- Binder, V., Bergum, B., Jaisson, S., et al. Impact of fibrinogen carbamylation on fibrin clot formation and stability. Thromb. Haemost. 117(5), 899-910 (2017).
- 3. Gajjala, P.R., Fliser, D., Speer, T., et al. Emerging role of post-translational modifications in chronic kidney disease and cardiovascular disease. Nephrol. Dial. Transplant. **30(11)**, 1814-1824 (2015).
- El-Gamal, D., Holzer, M., Gauster, M., et al. Cyanate is a novel inducer of endothelial ICAM-1 expression. Antioxid. Redox Signal. 16(2), 129-137 (2012).
- Shi, J., Knevel, R., Suwannalai, P., et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc. Natl. Acad. Sci. USA 108(42), 17372-17377 (2011).

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