

PRODUCT INFORMATION

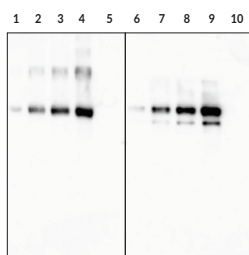


Anti-Carbamylation (Homocitrulline) Polyclonal Antibody Item No. 22428

Overview and Properties

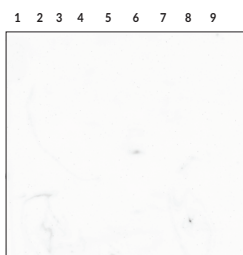
Contents:	This vial contains 500 µl of hapten affinity-purified polyclonal antibody.
Synonym:	pan-Carbamylation
Immunogen:	Carbamyated protein
Cross Reactivity:	(+) Carbamyated proteins; (-) Citrullinated proteins
Species Reactivity:	Species Independent
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	≥3 years
Storage Buffer:	PBS, pH 7.2, with 50% glycerol 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 µg of antibody per IP test. Other applications were not tested, therefore optimal working concentration/dilution should be determined empirically.

Images



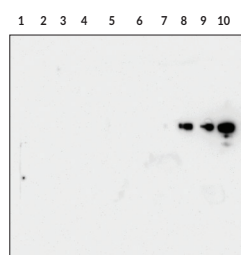
Western blot using the Anti-Carbamylation (Homocitrulline) Polyclonal Antibody.

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)



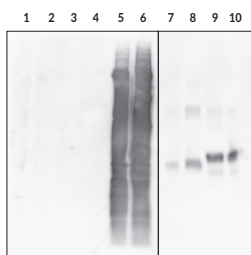
Western blot using the Anti-Carbamylation (Homocitrulline) Polyclonal Antibody.

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 (100 ng)
Lane 9: Alpha Enolase (200 ng)



Immunoprecipitation (IP) with the Anti-Carbamylation (Homocitrulline) Polyclonal Antibody. Antibodies were coupled to Protein A resin (5 µg 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 (α chain) Monoclonal Antibody (Clone 6D6) (Item No. 18793).

Lane 1: No Antibody; Carbamylated Fibrinogen (2 µl IP reaction)
Lane 2: No Antibody; Carbamylated Fibrinogen (10 µl IP reaction)
Lane 3: Negative Control Polyclonal Antibody; Carbamylated Fibrinogen (2 µl IP reaction)
Lane 4: Negative Control Polyclonal Antibody; Carbamylated Fibrinogen (10 µl IP reaction)
Lane 5: Anti-Carbamylation Polyclonal Antibody; Unmodified Fibrinogen (2 µl IP reaction)
Lane 6: Anti-Carbamylation Polyclonal Antibody; Unmodified Fibrinogen (10 µl IP reaction)
Lane 7: Anti-Carbamylation Polyclonal Antibody; Carbamylated Fibrinogen (2 µl IP reaction)
Lane 8: Anti-Carbamylation Polyclonal Antibody; Carbamylated Fibrinogen (10 µl IP reaction)
Lane 9: Fibrinogen (+ Western blot control) (10 ng)
Lane 10: Fibrinogen (+ Western blot control) (50 ng)



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)

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

SAFETY 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
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PRODUCT INFORMATION



Description

Carbamylation is a non-enzymatic and irreversible post-translational protein modification whereby cyanate/isocyanic acid adds a carbamoyl group to the ϵ -amino of lysine side chains, converting lysine to the non-standard amino acid homocitrulline.^{1,2} Cyanate is formed primarily from the breakdown of urea but can also be formed from the oxidation of thiocyanate by myeloperoxidase in the presence of hydrogen peroxide at sites of inflammation, such as atherosclerotic tissues.¹⁻³ LDL can be carbamylated and has atherogenic properties, disrupting LDL receptor binding and increasing vascular smooth muscle cell proliferation and endothelial cell death.^{3,4} Protein carbamylation and carbamylated LDL levels are increased in the plasma of patients with disorders resulting in high levels of urea, such as chronic and end-stage renal diseases.^{2,4} Furthermore, the levels of plasma protein-bound homocitrulline positively correlate with the frequency of, and mortality from, major adverse cardiac events and predict cardiovascular disease risk.³ Carbamylated proteins can also induce an immune response leading to the production of anti-homocitrulline autoantibodies, which have been found at higher levels in individuals with rheumatoid arthritis and are positively associated with the degree of joint damage.⁵ Cayman's Anti-Carbamylation (Homocitrulline) Polyclonal Antibody can be used for ELISA, immunoprecipitation (IP), and Western blot (WB) applications. The antibody recognizes carbamylated proteins and does not detect citrullinated proteins.

References

1. Stark, G.R., Stein, W.H., and Moore, S. Reactions of cyanate present in aqueous urea with amino acids and proteins. *J. Biol. Chem.* **235(11)**, 3177-3181 (1960).
2. Long, J., Vela Parada, X., and Kalim, S. Protein carbamylation in chronic kidney disease and dialysis. *Adv. Clin. Chem.* **87**, 37-67 (2018).
3. Wang, Z., Nicholls, S.J., Rodriguez, E.R., *et al.* Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat. Med.* **13(1)**, 1176-1184 (2007).
4. Ok, E., Basnakian, A.G., Apostolov, E.O., *et al.* Carbamylated low-density lipoprotein induces death of endothelial cells: A link to atherosclerosis in patients with kidney disease. *Kidney Int.* **68(1)**, 173-178 (2005).
5. 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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