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PRODUCT INFORMATION



Nitrotyrosine Affinity Sorbent

Item No. 389549

Contents:	This vial contains 200 µg of anti-Nitrotyrosine Monoclonal Antibody (Item No. 189542) coupled to protein A agarose. The product is supplied as a 50% slurry in PBS, pH 7.2, with 0.02% sodium azide.
Storage:	4°C
Stability:	≥1 year

Laboratory Procedures

The Nitrotyrosine Affinity Sorbent is designed for immunoprecipitation of nitrated proteins from biological samples. Immunoprecipitation is an effective way to concentrate nitrated proteins for subsequent detection by a different anti-nitrotyrosine antibody, such as Cayman's Nitrotyrosine Polyclonal Antibody (Item No. 189540) by Western blot analysis. Alternatively, an antibody against a specific protein of interest can be used to detect a particular nitrated protein.

A typical procedure for immunoprecipitation is provided as follows:

1. Dilute the protein sample (cell lysate, tissue homogenate, plasma, etc.) to approximately 1 mg/ml in TBS or PBS in a microcentrifuge tube.
2. Add 20-40 µl of gel slurry to 0.5-1 ml (0.5-1 mg protein) and incubate 1-2 hours at room temperature or 4°C overnight with occasional mixing or gentle rocking.
3. Collect the beads by brief centrifugation (<1 minute) in a microcentrifuge. Carefully drain or pipette off the supernatant and wash the beads three times with TBS or PBS.
4. Resuspend the beads in 25-50 µl of 1X Laemmli sample buffer and boil for five minutes. (Alternatively, the samples can be frozen for later use).
5. Briefly centrifuge the sample to pellet the beads. The supernatant can now be directly used for SDS-PAGE.

Description

Nitrotyrosine is a post-translational modification that is formed by the nitration of tyrosine.¹ It exists in a free or protein-bound form and is commonly used as a marker of nitrosative or oxidative stress.² Nitrotyrosine residues have been found in a variety of proteins, including LDL, surfactant protein A, angiotensin II, and human and bovine serum albumin.³ Nitrotyrosine levels are increased in the affected tissues of numerous pathological conditions, including atherosclerosis, cancer, ulcerative colitis, Alzheimer's disease, and Parkinson's disease.³ Autoantibodies that recognize nitrotyrosinated proteins are increased in the synovium of patients with rheumatoid arthritis and are positively correlated with joint and tendon inflammation.¹ Cayman's Nitrotyrosine Affinity Sorbent is designed for immunoprecipitation (IP) of nitrated proteins from biological samples. The nitrotyrosine affinity sorbent consists of Cayman's Nitrotyrosine Monoclonal Antibody (Item No. 189542) coupled to protein A agarose. This is an effective way to concentrate nitrated proteins for subsequent detection by a different anti-nitrotyrosine antibody by Western blot (WB).

References

1. Smallwood, M.J., Nissim, A., Knight, A.R., *et al.* Oxidative stress in autoimmune rheumatic diseases. *Free Radic. Biol. Med.* **125**, 3-14 (2018).
2. Teixeira, D., Fernandes, R., Prudêncio, C., *et al.* 3-Nitrotyrosine quantification methods: Current concepts and future challenges. *Biochimie* **125**, 1-11 (2016).
3. Oldreive, C. and Rice-Evans, C. The mechanisms for nitration and nitrotyrosine formation *in vitro* and *in vivo*: Impact of diet. *Free Radic. Res.* **35**(3), 215-231 (2001).

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.

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