Methionine Sulfoxide Immunoblotting Kit

Item No. 600160

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity	Storage
600166	Methionine Sulfoxide Polyclonal Antibody	1 vial	-20°C
600162	Methionine Sulfoxide Protein Positive Control	1 vial	-20°C
600163	Methionine Sulfoxide IgG-Blocking Reagent	1 vial	4°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

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.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as listed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Electrophoresis equipment; SDS-PAGE gels, buffers, etc.
2. Electrotransfer equipment; nitrocellulose or PVDF membranes, buffers etc.
3. A source of deionized water
4. 2X reducing or non-reducing sample buffer

INTRODUCTION

Background

Protein methionine sulfoxide (MetO) is a reversible oxidative modification that occurs by exposure of proteins methionine residues to reactive oxygen species (ROS).[1,2] Methionine oxidation can alter the functions of the modified proteins and if not reversed by methionine sulfoxide reductases can be further oxidized to methionine sulfone, an irreversible modification.[3] Methionine oxidation may occur as a by-product of phagocytotic oxidative bursts, normal mitochondrial respiration, and may also occur from environmental chemical exposures.[1,4,5] The overabundance of methionine sulfoxidation is implicated in age-related diseases.[4] Cellular protein MetO levels may be influenced by the decrease or loss of methionine sulfoxide reductase (MSR) activity or by an overabundance of ROS leading to increased levels of dysfunctional proteins.[5,6]

About This Assay

Cayman's MetO Immunoblotting Kit contains reagents needed for the immunochemical detection of proteins containing MetO residues by western blotting. MetO-containing samples of interest include those from cell or tissue lysates as well as semi-pure or purified proteins. Samples may be prepared with reducing or non-reducing sample buffer prior to SDS-PAGE and tested along side one SDS-PAGE well designated for the provided positive control. The MetO polyclonal antibody was isolated from rabbit serum generated after immunization with an oxidized peptide from the corn protein DZS18, which is unusually rich in methionine.[2] This polyclonal antibody is specific for protein MetO and has minimal cross-reactivity with methionine sulfone.

PRE-ASSAY PREPARATION

Positive Control Preparation for Electrophoresis

The MetO Immunoblotting Kit provides a positive control protein extensively modified with oxidized methionine. To prepare the positive control, dissolve the lyophilized powder in the MetO protein positive control (Item No. 600162) vial with 100 µl of sample buffer **without** reducing agent (*i.e.*, β-mercaptoethanol or dithiothreitol). Exposure to boiling water is not required for preparation. The positive control allows internal quality control of antibody function for each of ten possible western blots. The positive control band is observed as a thick 90 kDa band when the antibody is functioning properly. There is enough positive control for 10 lanes. Store the positive control at -20°C for up to one year.

Polyclonal Antibody Preparation for Immunoblotting

The MetO polyclonal antibody (Item No. 600166) is protein A purified rabbit IgG in 500 µl of PBS, pH 7.2, with 50% glycerol, 0.1% BSA, and 0.02% sodium azide. Use the antibody at 1:200 dilution as a primary antibody for detection of proteins containing MetO. There is enough antibody for 10 blots.

IgG-Blocking Reagent Preparation

The MetO IgG-blocking reagent (Item No. 600163), contains MetO (10 mg/vial) and is provided to confirm the specificity of the MetO polyclonal antibody. Resuspend the MetO IgG-blocking reagent (powder) with 200 µl PBS or TBS buffer and gentle agitation. Store the blocking reagent stock solution at 4°C for up to six months.

ASSAY PROTOCOL

Performing the Assay

Experimental Samples

Prepare protein-containing samples (clarified lysates, semi-pure or pure proteins, plasma or CSF) with an equal volume of reducing or non-reducing buffer and gentle mixing. Alternatively 5X sample buffer may be used if already available.

Membrane Blocking

Immediately after proteins have been transferred from the gel(s) to either nitrocellulose or PVDF membrane, place the membrane in 20 ml of blocking solution (0.5% non-fat dry milk in TBS, pH 7.4, or PBS buffer). Incubate for two hours at room temperature with gentle shaking or overnight at 4°C.

Wash 1

Pour off the blocking solution and wash the membrane three times with TBS, pH 7.4, containing 0.1% Polysorbate 20 for five minutes per wash.

Primary Antibody Incubation

Incubate the membrane with the primary antibody at 1:200 dilution in 0.5% non-fat dry milk in TBS, pH 7.4, or a similar buffer. Membranes should be incubated in the primary antibody for at least one hour. Increased sensitivity may be achieved by incubating for longer periods of time (at room temperature or 16-18 hours at 4°C).

Wash 2

Wash the membrane at least three times; same as wash 1.

Secondary Antibody Incubation

Dilute an enzyme linked anti-rabbit secondary antibody (*i.e.*, Cayman's goat anti-rabbit IgG-HRP, Item No. 10004301) according to the manufacturer specifications in blocking buffer. Incubate on the membrane with gentle shaking for one hour at room temperature.

Wash 3

Wash the membrane at least 3-5 times (5 minutes/wash), using the same wash buffer as in previous washes.

Development

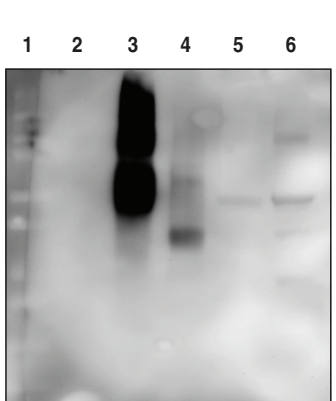
Develop the blot using either ECL or a colorimetric technique according to the manufacturer specifications.

Optional Technique: Negative Control Immunoblot

Add 100 µl of the MetO IgG-blocking reagent and 50 µl of undiluted MetO antibody into a microfuge tube, mix gently, and incubate overnight at 4°C (a one hour incubation at room temperature is not sufficient for complete blocking). Dilute the negative control or "blocked" antibody to 10 ml in TBS or PBS containing 0.5-1.0 % non-fat dry milk and use for blotting in parallel with a second identical blot using the MetO polyclonal antibody alone (also at 1:200 working dilution).

ANALYSIS

Performance Characteristics



Lane 1: MW stds
Lane 2: BSA (1.5 µg)
Lane 3: BSA-MetO protein Postive Control (1.5 µg)
Lane 4: Oxidized DZS18 protein standard (0.6 µg)
Lane 5: Mouse brain lysate, no H_2O_2 treatment, 2 hours, 37°C (50 µg)
Lane 6: Mouse brain lysate, 100 mM H_2O_2 treated, 2 hours, 37°C (50 µg)

Figure 1. Antibody specificity determined by western blot employing BSA conjugates or peroxide treated and control tissue lysates. MetO polyclonal antibody (1:200 dilution) was incubated on the membrane for one hour at room temperature and washed. Cayman's goat anti-rabbit IgG-HRP secondary (1:4,000) was then incubated for one hour at room temperature followed by washes and standard ECL development.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No detection of positive control	Possible loss of antibody activity (either primary or anti-rabbit secondary antibody)	Review and repeat the immunoblotting method and contact Technical Support if no development occurs

References

1. Stadtman, E.R., Van Remmen, H., Richardson, A., *et al.* Methionine oxidation and aging. *Biochim. Biophys. Acta* **1703**, 135-140 (2005).
2. Oien, D.B., Canello, T., Gabizon, R., *et al.* Detection of oxidized methionine in selected proteins, cellular extracts and blood serums by novel anti-methionine sulfoxide antibodies. *Arch. Biochem. Biophys.* **485**, 35-40 (2009).
3. Moskovitz, J., Jenkins, N.A., Gilbert, D.J., *et al.* Chromosomal localization of the mammalian peptide-methionine sulfoxide reductase gene and its differential expression in various tissues. *Proc. Natl. Acad. Sci. USA* **93**, 3205-3208 (1996).
4. Berlett, B.S. and Stadtman, E.R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.* **272(33)**, 20313-20316 (1997).
5. Cabreiro, F., Picot, C.R., Perichon, M., *et al.* Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage. *J. Biochem.* **283(24)**, 16673-16681 (2008).
6. Levine, R.L., Mosoni, L., Berlett, B.S., *et al.* Methionine residues as endogenous antioxidants in proteins. *Proc. Natl. Acad. Sci. USA* **93**, 15036-15040 (1996).

NOTES

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. This document is copyrighted. All rights are reserved.

This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.