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SNOB 1 reagent and negative control

Cat. No. 3879 & 3880
For research use only

INTRODUCTION

S-NO Binding (SNOB) Reagents provide a convenient and sensitive method for the direct visualization, or proteomic characterization, of S-nitrosylated proteins. SNOB reagents modify S-nitrosothiol groups on proteins in a single chemical step which is compatible with physiological conditions. These reagents are suitable for the direct visualization of S-nitrosylation using SDS-page and Western blot techniques, as well as the proteomic analysis of S-nitrosylation sites using mass spectroscopy.

PRODUCTS

Item	***Cat. No.***	***Format***
SNOB 1 reagent	3879	10 mg (dry powder)
SNOB 1 control	3880	10 mg (dry powder)

STORAGE

Upon receipt, store the solid compound at +4^{o}C.
Prepared stock solutions should be stored aliquoted at -20^{o}C and protected from light. Stock solutions should be discarded after 1 month. Wherever possible solutions should be made up and used on the same day.

KEY REAGENTS REQUIRED BUT NOT PROVIDED

- Dimethyl sulfoxide (DMSO) – available from Tocris (see ***Cat. No. 3176****)*
- *For Western blotting:* Avidin, streptavidin or neutravidin-conjugated to horseradish peroxidase (HRP) or a fluorescent tag

PREPARATION OF SNOB 1 REAGENT STOCK SOLUTION (X20)

Dissolve 3 mg SNOB 1 Reagent (**Cat. No. 3879**) in 100 µl of DMSO, and then dilute to a final volume of 1 ml with phosphate buffered saline (PBS) or other suitable buffer solution for live cell experiments (900 µl), to produce a stock solution (x20) of SNOB Reagent. If required, a stock solution (x20) of SNOB 1 control (**Cat. No. 3880**) can be prepared using the same procedure.

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GENERAL PROCEDURE FOR VISUALIZATION OF S-NITROSYLATED PROTEINS USING WESTERN BLOTTING TECHNIQUE

1A. Labeling of cell surface S-nitrosylation of live cells:
Dilute SNOB 1 Reagent stock solution (x20) in PBS or other suitable physiological buffer solution to give x1 working solution. Remove cell culture medium and incubate cells with diluted SNOB Reagent solution for up to 30 minutes at 37˚C in light limited conditions. Remove diluted SNOB reagent solution by washing adherent cells or centrifugation/re-suspension of suspension cells three times in PBS or buffer. Prepare cell lysates or membranes by standard procedures.

1B. Labeling of S-nitrosylated proteins in isolated cell lysates:
Dilute stock SNOB 1 Reagent solution (x20) into lysate sample to give a x1 working solution and incubate at 37˚C for up to 30 minutes in light limited conditions.

2. Separate proteins by electrophoresis and transfer proteins from the gel onto a nitrocellulose or PVDF membrane.

3. Block membrane with 5% (w/v) bovine serum albumin (BSA) overnight at 4˚C (***do not*** use blocking reagents containing milk since endogenous biotin will interfere with the detection procedures).

4. Detect SNOB 1 reagent and control with commercial avidin, streptavidin or neutravidin-conjugated to HRP or a fluorescent tag. Optimize the dilution, reaction time and incubation temperature referring to the recommendations by the manufacturer.

5. Visualize labeled proteins using an appropriate detection system (e.g. Tocris ***Cat. No. 3187***)

DIAGRAM OF OVERALL PROCEDURE