



COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual PVDF Blocking Reagent for Can Get Signal® 0810

F1029K

PVDF Blocking Reagent

for *Can Get Signal*®

NYPBR01 500 ml
Store at 4 °C.

Contents

-
- [1] **Introduction**
 - [2] **Components**
 - [3] **Protocol**
 - [4] **Application data**
 - [5] **Related products**
-

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions and follow safety guidelines while using this kit.



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[1] Introduction

Description

PVDF Blocking Reagent for *Can Get Signal*[®] is a high performance blocking reagent optimized for Western blot. The reagent consists of a synthesized polymer, with no protein components. The reagent can be used efficiently with *Can Get Signal*[®] Immunoreaction Enhancer Solution.

Features

- PVDF Blocking Reagent for *Can Get Signal*[®] has been optimized for use together with *Can Get Signal*[®] Immunoreaction Enhancer Solution (Code No. NKB-101) for Western blot analysis.
- The reagent is suitable for detection of phosphorylated proteins, because it does not contain any protein components.
- The reagent minimizes the masking effects of low signal intensities, whereas conventional blocking reagents (*e.g.*, non-fat milk and gelatin) can mask Western blot protein signals.

[2] Components

This reagent should be stored at 4°C.

PVDF Blocking Reagent for *Can Get Signal*[®] 500 ml

Notes:

- The reagent contains 0.1% sodium azide.

[3] Protocol

PVDF Blocking Reagent should be used directly to block non-specific protein binding on Western blots.

The standard protocol is as follows:

- (1) Wash transferred membranes in Wash Buffer (*e.g.*, TBS-T: TBS/0.1% Tween 20) for 5 min while shaking.
- (2) Replace Wash Buffer with PVDF Blocking Reagent for *Can Get Signal*[®].
- (3) Incubate at RT-37°C for 1 hr, or at 4°C overnight.
- (4) Rinse the membrane in Wash Buffer.
- (5) Wash the membrane in Wash Buffer for 15 min, and twice for 5 min per wash while shaking.
- (6) Proceed to the primary antibody reaction step.

Notes:

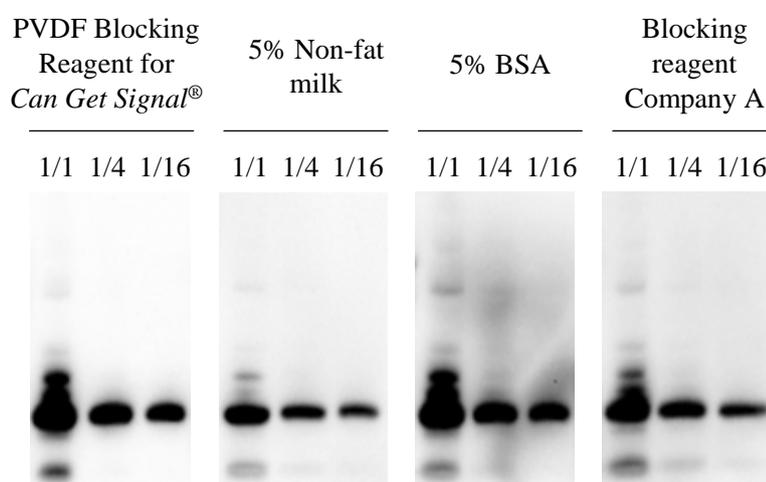
- PVDF Blocking Reagent for *Can Get Signal*[®] can be applied to PVDF (polyvinylidene difluoride) and nitrocellulose membranes used for Western blot analysis. The reagent works more efficiently on PVDF membranes.
- This reagent has been optimized for Western blot analysis with *Can Get Signal*[®] Immunoreaction Enhancer Solution. Although this reagent can be used for conventional Western blot analysis, blocking efficiency may be decreased.
- Because this reagent contains 0.1% sodium azide, residual sodium azide may inhibit HRP activity. Therefore, the washing step should not be skipped after the blocking step.

[4] Application data Example 1

<Assay conditions>

SDS-PAGE: 8-16% polyacrylamide gel, 15 mA × 90 min
 Transfer: 0.8 mA/cm² at RT for 60 min (semi-dry method)
 Blocking: RT for 60 min
 Sample: HeLa cell lysates 2 × 10⁴ cells/well (1/1), 4ⁿ dilution (1/4, 1/16)
 Primary antibody: rabbit anti-ERK2 (C-14) antibody (0.1 ng/μl) in *Can Get Signal*[®] Solution 1
 Secondary antibody: HRP-conjugated anti-rabbit IgG antibody (0.02 ng/μl) in *Can Get Signal*[®] Solution 2
 Detection reagent: ECL Plus (GE Healthcare)

<Result>



PVDF Blocking Reagent for *Can Get Signal*[®] successfully increased protein signal intensity and reduced non-specific background staining.

Example 2

<Assay conditions>

SDS-PAGE: 8-16% polyacrylamide gel, 15 mA × 90 min

Transfer: 0.8 mA/cm² at RT for 60 min (semi-dry method)

Blocking: RT for 60 min

Sample: HeLa cell lysates 2 × 10⁴ cells/well (1/1), 4ⁿ dilution (1/4, 1/16)

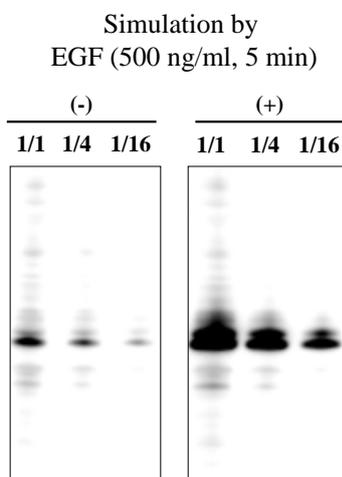
Cells were stimulated with EGF.

Primary antibody: mouse anti-p-ERK2 (E-14) monoclonal antibody (0.2 ng/μl) in *Can Get Signal*[®] Solution 1

Secondary antibody: HRP-conjugated anti-mouse IgG antibody (0.01 ng/μl) in *Can Get Signal*[®] Solution 2

Detection reagent: ECL Plus (GE Healthcare)

<Result>



The distinct bands (p-ERK1: 44 kDa, p-ERK2: 42 kDa) were successfully detected with minimal background staining.

[5] Related products

Product name	Package	Code No.
<i>Can Get Signal</i> [®] Solution 1 for primary antibody	250 ml each	NKB-101
Solution 2 for secondary antibody	50 ml each	NKB-101T
<i>Can Get Signal</i> [®] Solution 1 for primary antibody	250 ml	NKB-201
<i>Can Get Signal</i> [®] Solution 2 for secondary antibody	250 ml	NKB-301



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3

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