Print

PhosSTOP

Phosphatase Inhibitor Cocktail Tablets provided in *EASYpacks*

Cat. No. 04 906 845 001 10 tablets
Cat. No. 04 906 837 001 20 tablets

Version 03
Content version: December 2012
Store at +2 to +8°C

Please note: Alltough the tablets are not toxic, they are harmful. Do not eat/ingest the tablets.

1. What this Product Does

Contents

Each PhosSTOP tablet contains a proprietary blend of phosphatase inhibitors.

Storage and Stability

- If stored at +2 to +8°C, the tablets are stable through the expiration date printed on the label.
- If stored at +15 to +25°C, the tablets are stable for 3 months.
- A stock solution of one tablet solved in 1 ml water is stable for more than 1 month if stored at +2 to +8°C, or for at least 6 months if stored at −15 to −25°C.

Application

PhosSTOP is a proprietary blend of phosphatase inhibitors, formulated as a ready-to-use, quick-dissolving, water-soluble tablet.

The PhosSTOP Phosphatase Inhibitor Cocktail Tablets inhibit a broad spectrum of phosphatases such as acid and alkaline phosphatases, as well as serine/threonine (PP1, PP2A, and PP2B) and tyrosine protein phosphatases (PTP).

The PhosSTOP tablets protect proteins of cells and tissues of many different origins (*e.g.,* mammalian, insect, and plant) against dephosphorylation. They are well suited for purification and detection of phosphorylated proteins.

In addition, the inhibitor tablets can be used in buffers containing formalin for formalin fixation of tissue sections.

PhosSTOP tablets can also be used in combination with the cOmplete Protease Inhibitor Cocktail Tablets to simultaneously protect proteins against dephosphorylation and proteolytic degradation; both inhibitor tablets maintain their activity when they are used together.

2. How to Use this Product

Precautions

⚠ **Although the tablets are not toxic, they are harmful and should be handled as such. Do not eat/ingest the tablets.** Carefully push the tablet through the foil. Do not use your finger nail since the tablet may break.

Preparation of Working Solutions

Use one tablet to inhibit phosphatase activity in 10 ml extraction solution. If necessary, 2 tablets can be used for the same volume.

Preparation of 10× conc. Stock Solution

Dissolve one PhosSTOP tablet in 1 ml water or buffer via swirling. The stock solution is stable for more than 1 month if stored at +2 to +8°C, or for at least 6 months if stored at −15 to −25°C.

3. Results

Phosphatases are ubiquitous. Depending on species, cell or organ type and status of the particular cells used in the experiment, the spectrum and quantity of the different phosphatases vary significantly. Although each phosphatase class shows different substrate specificity, depending on the particular species, several isolated phosphatases have been tested in order to obtain some idea of the range of inhibition (Table 1).

Tab. 1: Inhibition of phosphatase activity of isolated phosphatases. For each assay a different phosphorylated peptide was used. Released phosphate was detected via a malachite green assay. The percentage given in the table is inhibitory efficiency. The final concentration of the dissolved PhosSTOP tablet in each assay was 1×.

Phosphatases	Phosphatase Activity (U/10 ml)	% Inhibition after 15 min incubation (1 tablet per 10 ml)
Calf Alkaline Phosphatase	140 U	98.4%
Potato Acidic Phosphatase	2 U	93.7%
Human Acidic Phosphatase	640 U	99.5%
Rabbit PP1	200 U	98.6%
Human PP2A	500 U	94.4%
Human PTP	500 U	96.7%

In addition, PhosSTOP tablets were used to inhibit different phosphatases in various types of cell extracts. The inhibitory efficiency of PhosSTOP was evaluated for alkaline (AP) and acid (SP) phosphatases, as well as for serine/threonine (PP1 and PP2A) and tyrosine protein phosphatases (PTP) (Table 2).

Tab. 2: Inhibition of phosphatase activity in different cell extracts. For each assay a different phosphorylated peptide was used. Released phosphate was detected via a malachite green assay. The percentage given in the table is inhibitory efficiency. The final concentration of the dissolved PhosSTOP tablet in each assay was 1×.

	AP	SP	PP1	PP2A[1]	PTP[1]
A 431 lysate[3]	100%	88.5%	98.2%	80.2%	67.1%[1]
COS lysate[3]	100%	100%	95.8%	52.3%[1]	68.1%[1]
Maize extract[2]	100%	69.0%	97.8%	72.2%	89.9%
Tabacco extract[2]	93.0%	70.2%	96.8%	100%	96.0%
Insect cell lysate[3]	100%	86.8%	94.6%	10.9%[1]	50.2%[1]

[1] Other enzymes in the cell extract may interfere with the assay results.

[2]Lysis via P-PER Plant Protein Extraction Kit (Pierce)

[3]Lysis via Lysis-M solution from cOmplete Lysis-M Kit* (Roche Applied Science).

The monitoring of phosphatase activity was complemented by running Western Blot experiments using *e.g.* phosphoserine-specific antibodies (Fig. 1).

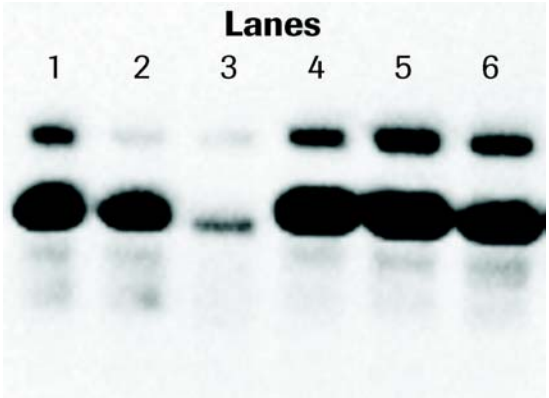

Fig. 1: Western blot of an insect cell lysate. Detection via anti-phosphoserine antibodies.
Lanes 1 - 3: Incubation without PhosSTOP inhibiton for 0, 3, and 24 hours at +15 to +25°C.
Lanes 4 - 6: Incubation with PhosSTOP inhibition for 0, 3, and 24 hours at +15 to +25°C.
Development of Western Blot after blotting: blocking with 1× solution of Blocking Reagent* in TBST, incubation with anti-phosphoserine/threonine antibody (BD Bioscience, Cat. No. 612549, 1:1000 dilution) in blocking solution. Incubation with secondary antibody (anti mouse-Ig, Chemicon, Cat. No. AP326P, 1:1000 dilution) in blocking solution. Detection using Lumi-Light^{PLUS} Western Blotting Substrate *.

PhosSTOP Tablets can also be used to prevent dephosphorylation in formalin fixed paraffin embedded (FFPE) tissue sections (Fig. 2).

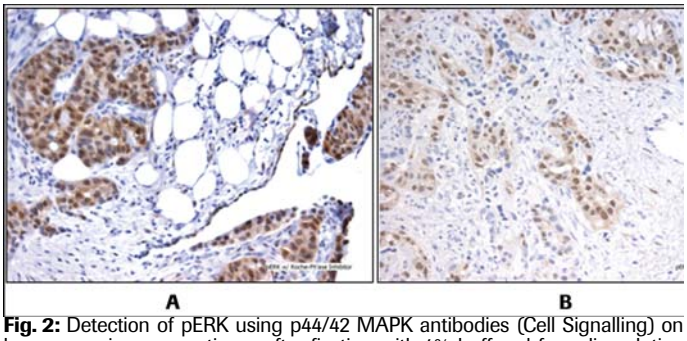

Fig. 2: Detection of pERK using p44/42 MAPK antibodies (Cell Signalling) on human ovarian cancer tissue after fixation with 4% buffered formalin solution with addition of PhosSTOP Tablet (A) in comparison to no addition of PhosSTOP Tablet (B). In addition, it was demonstrated that other non-phosphorylated markers could be detected equally well on formalin fixed tissue section with and without addition of PhosSTOP Tablet.

4. Additional Information on this Product

PhosSTOP inhibitors were evaluated for their effect on protein assays. The inhibitors show almost no influence on BCA (see Figure 3) or Bradford protein assays.

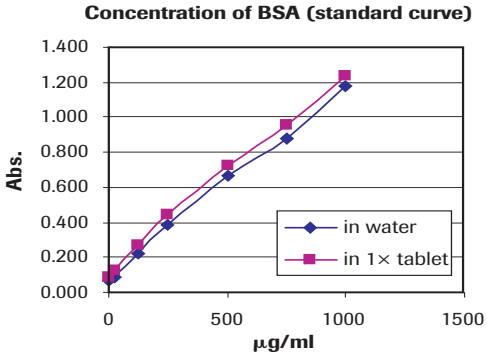

Fig. 3: Determination of Bovine Serum Albumin (BSA) protein concentration using the BCA assay (Pierce) with and without addition of PhosSTOP Tablet.

However, we recommend running control experiments in order to ensure that no cooperative interference (tablet with components of the particular buffer) occurs.

Quality Control

The inhibition of acidic and alkaline phosphatase as well as Serine/Threonine- and Tyrosine phosphatases is tested.

References

For a review of protein phosphatases see: Methods in Enzymology vol. 366, Protein phosphatases; eds. S. Klumpp and J. Krieglstein, Elsevier Academic Press 2003.

5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Symbol	Description
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered Instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

5.2 Changes to Previous Version

Editorial and layout changes.

5.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Site:

www.roche-applied-science.com/phosphataseinhibitor

Product	Pack Size	Cat No.
PhosSTOP	10 tablets in foil blisters (for 10 ml each)	04 906 845 001
PhosSTOP	20 tablets in foil blisters (for 10 ml each)	04 906 837 001
cOmplete ULTRA Protease Inhibitor Cocktail Tablets		
cOmplete ULTRA Tablets, Mini, EDTA-free, *EASYpack*	30 tablets in foil blisters (for 10 ml each)	05 892 791 001
cOmplete ULTRA Tablets, EDTA-free, glass vials	2 ×10 tablets in glass vials (for 50 ml each) 6 ×10 tablets in glass vials (for 50 ml each)	05 892 953 001 05 892 953 001
cOmplete ULTRA Tablets, Mini, with EDTA, *EASYpack*	30 tablets in foil blisters (for 10 ml each)	05 892 970 001
cOmplete ULTRA Tablets, with EDTA, glass vials	2 ×10 tablets in glass vials (for 50 ml each) 6 ×10 tablets in glass vials (for 50 ml each)	05 892 988 001 06 538 304 001
cOmplete Protease Inhibitor Cocktail Tablets in foil blisters:		
cOmplete	20 tablets in foil blisters (for 50 ml each)	04 693 116 001
cOmplete, Mini	30 tablets in foil blisters (for 10 ml each)	04 693 124 001
cOmplete, EDTA-free	20 tablets in foil blisters (for 50 ml each)	04 693 132 001
cOmplete, Mini, EDTA-free	30 tablets in foil blisters (for 10 ml each)	04 693 159 001
Western Blotting Reagents and Kits		
Lumi-Light^{PLUS} Western Blotting Kit	1 kit (1,000 cm^2)	12 015 218 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml	12 015 196 001
Lumi-Light Western Blotting Substrate	400 ml	12 015 200 001
BM Blue POD Substrate, precipitating	100 ml	11 442 066 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set (1000 cm^2) 1 set (4000 cm^2)	11 500 708 001 11 500 694 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches; 20.3 × 25.4 cm) 100 films (7.1 × 9.4 inches; 18 × 24 cm)	11 666 657 001 11 666 916 001
Western Blotting Reagent, Solution	100 ml (10 blots, 100 cm^2 6 ×100 ml (60 blots, 100 cm^2)	11 921 673 001 11 921 681 001
PVDF Western Blotting Membranes	1 roll	03 010 040 001
Tween 20	5 × 10 ml	11 332 465 001
BSA, Fraction V, fatty acid free	50 g	10 775 835 001

5.4 Trademarks

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5.5 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

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