

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



PhosSTOP

 **Version: 05**
Content Version: June 2021

Phosphatase Inhibitor Cocktail Tablets provided in *EASYPacks*.

Cat. No. 04 906 845 001	10 tablets for 10 ml each
Cat. No. 04 906 837 001	20 tablets for 10 ml each

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	PhosSTOP, <i>EASYpack</i> , Phosphatase Inhibitor Cocktail Tablets	<ul style="list-style-type: none"> Individually packaged tablets Each tablet contains a proprietary blend of phosphatase inhibitors. 	04 906 845 001	10 foil blister packs, 1 tablet each
			04 906 837 001	20 foil blister packs, 1 tablet each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	PhosSTOP, <i>EASYpack</i> , Phosphatase Inhibitor Cocktail Tablets	Store at +2 to +8°C. Store at +15 to +25°C for 3 months.

1.3. Additional Equipment and Reagent required

For preparation of stock solution

- Double-distilled water or buffer

1.4. Application

PhosSTOP is a proprietary blend of phosphatase inhibitors, formulated as a ready-to-use, quick-dissolving, water-soluble tablet. It can be used for several applications:

- 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).
- Protect proteins of cells and tissues of many different origins, such as mammalian, insect, and plant, against dephosphorylation.
- 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.
- Use 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

2.1. Before you Begin

Working Solution

⚠ *Carefully push the tablet through the foil packaging using the base of your thumb (not fingernail) to prevent the breakage of tablets.*

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 10x-concentrated stock solution

Dissolve one PhosSTOP tablet in 1 ml double-distilled water or buffer.

i *The stock solution is stable for 1 month at +2 to +8°C or 6 months at -15 to -25°C.*

2.2. Parameters

Working Concentration

1 tablet per 10 ml extraction solution.

3. Results

Phosphatases are ubiquitous. Depending on species, cell or organ type, and status of the cells used, the spectrum and quantity of the different phosphatases vary significantly. Each phosphatase class shows different substrate specificity, depending on the particular species.

Typical values for the inhibition of phosphatase activity of isolated phosphatases are shown in the following table.

- For each assay, a different phosphorylated peptide was used.
- Released phosphate was detected via a malachite green assay.
- The percentage states the inhibitory efficiency.
- The final concentration of the dissolved PhosSTOP tablet in each assay was 1x.

Phosphatases	Phosphatase Activity [U/10 ml]	% Inhibition after 15 min incubation [1 tablet per 10 ml]
Calf Alkaline Phosphatase	140	98.4
Potato Acidic Phosphatase	2	93.7
Human Acidic Phosphatase	640	99.5
Rabbit PP1	200	98.6
Human PP2A	500	94.4
Human PTP	500	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), as shown in the following table.

Typical values for the inhibition of phosphatase activity in different cell extracts are shown in the table below.

- For each assay, a different phosphorylated peptide was used.
- Released phosphate was detected via a malachite green assay.
- The percentage states the inhibitory efficiency.
- The final concentration of the dissolved PhosSTOP tablet in each assay was 1x.

	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*.

The monitoring of phosphatase activity was complemented by running western blot experiments using, for example, phosphoserine-specific antibodies (Fig. 1).

3. Results

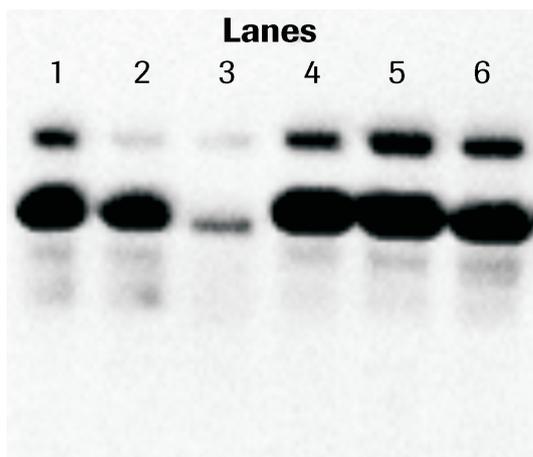


Fig. 1: Western blot of an insect cell lysate. Detection via anti-phosphoserine antibodies.

Lanes 1 to 3: Incubation without PhosSTOP, inhibition for 0, 3, and 24 hours at +15 to +25°C.

Lanes 4 to 6: Incubation with PhosSTOP, inhibition for 0, 3, and 24 hours at +15 to +25°C.

Development of western blot after blotting: blocking with 1x solution of Blocking Reagent in TBST, incubation with anti-phosphoserine/threonine antibody (BD Bioscience, Cat. No. 612549, 1:1,000 dilution) in blocking solution.

Incubation with secondary antibody (anti mouse-Ig, Chemicon, Cat. No. AP326P, 1:1,000 dilution) in blocking solution.

Detection using Lumi-Light^{PLUS} Western Blotting Substrate.

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

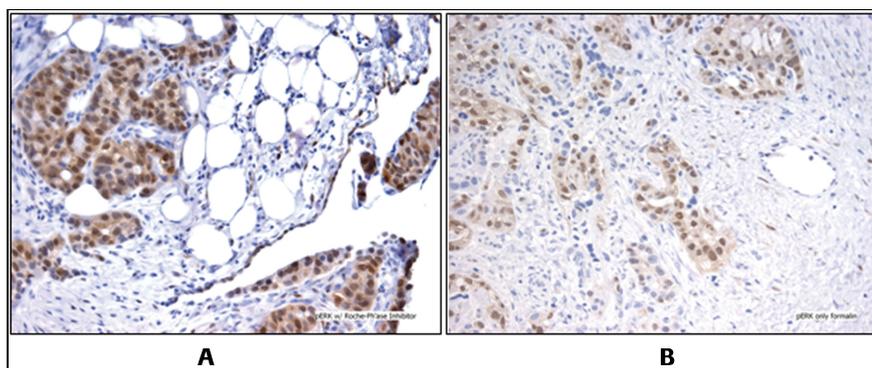


Fig. 2: Detection of pERK using p44/42 MAPK antibodies (Cell Signalling) on human ovarian cancer tissue after fixation with 4% buffered formalin solution with addition of PhosSTOP Tablet (A) in comparison to no addition of PhosSTOP Tablet (B). In addition, it was demonstrated that other non-phosphorylated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

PhosSTOP inhibitors were evaluated for their effect on protein assays. The inhibitors show almost no influence on BCA, as shown in Figure 3, or Bradford protein assays.

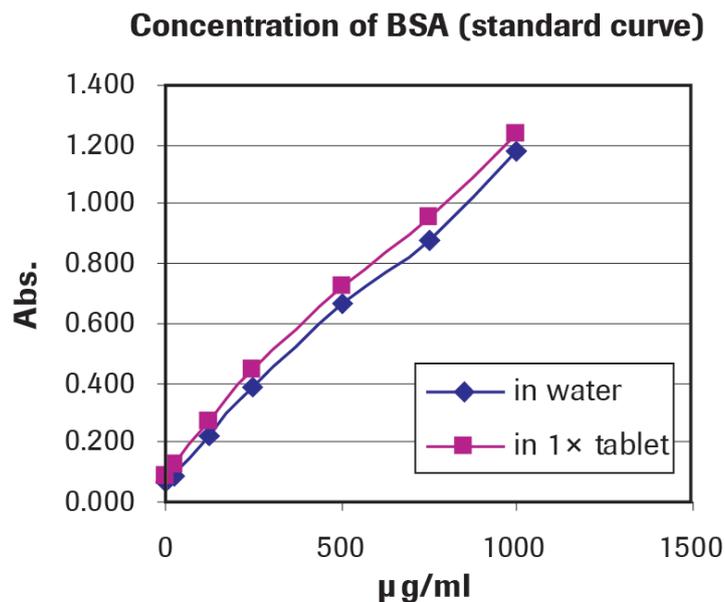


Fig. 3: Determination of Bovine Serum Albumin (BSA) protein concentration using the BCA assay (Pierce) with and without addition of PhosSTOP Tablet.

i Run control experiments to ensure that no cooperative interference (tablet with components of the particular buffer) occurs.

4. Additional Information on this Product

4.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

i *Information Note: Additional information about the current topic or procedure.*

⚠ Important Note: Information critical to the success of the current procedure or use of the product.

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.

Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
cOComplete Lysis-M	1 kit, lysis of up to 100 g of mammalian cells	04 719 956 001
cOComplete	20 tablets in a glass vial, for 50 ml each	11 697 498 001
	3 x 20 tablets in glass vials, for 50 ml each	11 836 145 001

5.4. Trademarks

PHOSSTOP is a trademark of Roche.

All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

