PhosSTOP – Phosphatase Inhibitor Cocktail Tablets

A New Reagent to Freeze Phosphorylation States

*Hans-Jürgen Rode and Erhard Fernholz**
Roche Applied Science, Penzberg, Germany
**Corresponding author: erhard.fernholz@roche.com*

Introduction

The regulation of many biological processes and pathways is accomplished by the formation and cleavage of phosphate esters. The phosphorylation of proteins is carefully balanced by an interplay of protein kinases and phosphatases. To understand these pathways, a great deal of scientific work is currently being carried out within the pharmaceutical industry and research institutes. An important step toward achieving this goal is to get an accurate view about general or specific phosphorylation status which requires a preservation of the phosphorylation pattern.

Phosphorylated proteins can be dephosphorylated by either nonspecific phosphatases (*e.g.*, alkaline phosphatase) or the more specific protein phosphatases like serine/threonine-specific, or tyrosine-specific phosphatases as well as dual-specific phosphatases.

Figure 1: Easy handling of PhosSTOP.

Here, we report about the performance of a new phosphatase inhibitor cocktail, called PhosSTOP. PhosSTOP is formulated as a ready-to-use tablet (Figure 1) since some of the phosphatase inhibitors are required in only nanomol amounts. It eliminates the time-consuming search for the right phosphatase inhibitors and the need to mix individual inhibitors. PhosSTOP contains inhibitors against acid and alkaline phosphatases, serine/threonine phosphatase classes (*e.g.*, PP1, PP2A and PP2B), tyrosine phosphatase (PTP) as well as dual-specific phosphatases.

One Phosphatase Inhibitor Cocktail Tablet is used for 10 ml lysate. However, since the solubility is very good, 2 or 3 tablets can be used for the same volume if necessary. Alternatively, a stock solution can be used if smaller volumes are desired. This stock solution is stable for more than a month at 4°C and can be frozen at -20°C for at least 6 months.

Results and Discussion

Phosphatases are ubiquitous. Depending on species, cell or organ type, and status of the particular cells used in the experiment, 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 first in order to obtain an idea about the range of inhibition (Table 1).

Table 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	Units (U/10 ml)	Inhibition (%)
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) (Table 2).

The monitoring of phosphatase activity was complemented by running western blot experiments with, for example, phosphoserine-specific antibodies (Figure 2).

PhosSTOP Tablets can also be used to prevent dephosphorylation in formalin-fixed paraffin-embedded (FFPE) tissue sections (Figure 3).

The inhibitors of PhosSTOP do not influence protein detection assays (*e.g.*, western blot or protein concentration determination assays such as BCA and Bradford).

Table 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]
A431 lysate[3]	100	88.5	98.2	80.2	67.1
COS lysate[3]	100	100	95.8	52.3	68.1
Maize extract[2]	100	69.0	97.8	72.2	89.9
Tobacco extract[2]	93.0	70.2	96.8	100	96.0
Insect cell lysate[3]	100	86.8	94.6	10.9	50.2

[1]Other enzymes in the cell extract may interfere with the assay.
[2]Lysis via P-PER Plant Protein Extraction Kit (Pierce)
[3]Lysis via Lysis-M solution from cOmplete Lysis-M Kit

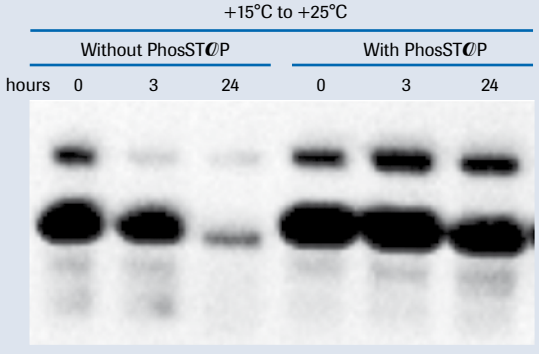

Figure 2: PhosSTOP protects proteins from phosphatases in insect cell lysates, as demonstrated on a western blot. Insect cell lysates were treated with PhosSTOP or left untreated, then incubated for the timepoints indicated above. Anti-Phosphoserine antibodies, a secondary antibody, and Lumi-Light[PLUS] Western Blotting Substrate were used to detect phosphorylated proteins.

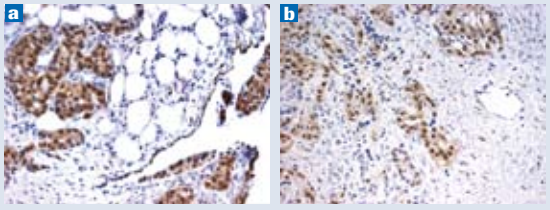

Figure 3: Protection of proteins' phosphorylation state in formalin-fixed paraffin-embedded (FFPE) human ovarian cancer tissue. Detection of pERK using p44/42 MAPK antibodies (cell signaling) on human ovarian cancer tissue after fixation with 4% buffered formalin solution with **(a)** addition of PhosSTOP in comparison to **(b)** no addition of PhosSTOP. In addition, it was demonstrated that other nonphosphorylated markers could be detected equally well on formalin fixed tissue section with and without addition of PhosSTOP.

Conclusions

PhosSTOP conveniently protects phosphorylated protein(s) against dephosphorylation. It shows an effective inhibition of a broad spectrum of phosphatases such as acid and alkaline phosphatases, serine/threonine phosphatase classes (*e.g.*, PP1, PP2A and PP2B), tyrosine phosphatase (PTP), and dual-specific phosphatases (data not shown).

The tablets inhibit phosphatases in a variety of sample materials including mammalian, insect, or plant cells. PhosSTOP is also well-suited for buffers containing formalin for the formalin-fixation of paraffin-embedded (FFPE) tissue sections. The non-toxic PhosSTOP tablets are easy-to-use and can be combined with cOmplete Protease Inhibitor Cocktail tablets. ■

Order your free sample of PhosSTOP or cOmplete at www.keep-it-easy.com.

To find out more about all our products to protect your proteins, and for ordering information, please visit www.roche-applied-science.com/phosphataseinhibitor.

Order INFO

Product	Pack Size	Cat. No.
PhosSTOP	10 tablets (for 10 ml each)	04 906 845 001
	20 tablets (for 10 ml each)	04 906 837 001
cOmplete, Mini	30 tablets (for 10 ml each)	04 693 124 001
cOmplete, Mini EDTA-free	30 tablets (for 10 ml each)	04 693 159 001
cOmplete Lysis-M	1 kit (20 extractions)	04 719 956 001
Lumi-Light[PLUS] Western Blotting Substrate	100 ml	12 015 196 001