

# MALDI-TOF Mass Analysis of Phosphorylated Molecules

## - Detection of Phosphate-binding Isotopic Zn<sup>2+</sup>-Phos-tag™ -

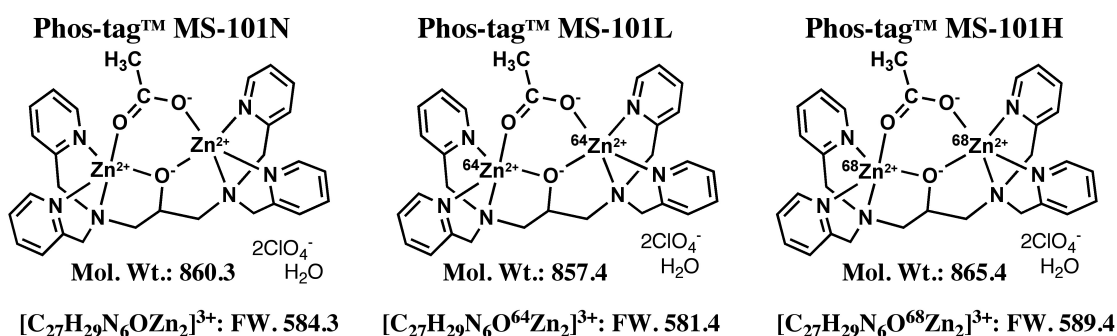
Ver.2 (2010/6)

### Introduction

Phosphorylation is a fundamental covalent post-translational modification that regulates the function, localization, and binding specificity of target proteins. Methods for determining the phosphorylation status of proteins (*i.e.*, phosphoproteomics) are thus very important with respect to the evaluation of diverse biological and pathological processes. In 2002, Prof. Koike's group (Hiroshima University) reported that a dinuclear metal complex (*i.e.*, 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olato dizinc(II) complex with a net charge of +3) acts as a selective phosphate-binding tag molecule, Phos-tag™ in an aqueous solution at a neutral pH (*e.g.*,  $K_d = 25$  nM for phenyl phosphate dianion,  $Ph-OPO_3^{2-}$ ). Since then, various methods for phosphoproteome research have been developed using Phos-tag™ derivatives. Here, we introduce a simple, rapid, and sensitive MALDI-TOF MS for analysis of phosphorylated molecules ( $ROPO_3^{2-}$ ) using an isotopic Zn<sup>2+</sup>-Phos-tag™ complex.

### Description of Phos-tag™ MS Analytical Kit

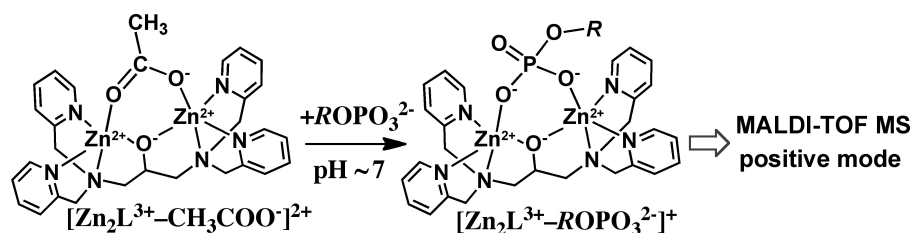
Phos-tag™ MS Analytical Kit (Phos-tag™ MS-101) provides a sensitive MALDI-TOF MS analysis of phosphorylated compounds ( $RO-PO_3^{2-}$ ) such as phospholipids and phosphopeptides in positive mode. The procedure for the MS analysis is almost the same as that for the general MALDI-TOF method. The products, Phos-tag™ MS-101N (natural abundant Zn), Phos-tag™ MS-101L (<sup>64</sup>Zn) and Phos-tag™ MS-101H (<sup>68</sup>Zn) are supplied as white crystalline zinc(II) complexes, which have no irritant effect on the skin. The Phos-tag™ MS-101 derivatives are non-radioactive and stable for 1 year at 4°C.



### Warning and Limitations

Phos-tag™ MS-101 is not for use in human diagnostic and the therapeutic procedures. Do not use internally or externally in human or animals. It's used only for research. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

### Principle of the MALDI-TOF MS using Zn<sup>2+</sup>-Phos-tag™



## Sample Solution

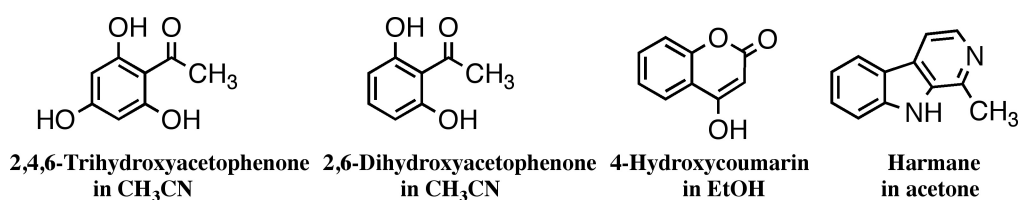
Because the Phos-tag™ molecule captures selectively a target phosphate in a pH range from 5 to 8, the buffer solution should be employed for the 1:1 complexation. If you are examining a sample mixture, it may be necessary to prepare the mixture with several different matrices (see the next section) in an appropriate buffer solution. The following buffer systems are recommended: 10 mM Tris-borate (pH 8), Good's buffers such as 10 mM HEPES/NaOH (pH 7.5), 10 mM TAPS/NaOH (pH 8.4) and 10 mM MES/NaOH (pH 6.2).

**Note:** Do not use inorganic phosphate for the buffer system, which is a competitive ligand to the Phos-tag™ molecule. A high salt concentration may interfere with sample ionization and increase the adduct peaks such as  $\text{Na}^+\text{-M}$  and  $\text{K}^+\text{-M}$ .

## Matrix Solution

The matrix molecule for MALDI-TOF MS plays a key role not only in the ionization process, but also in the complexation of a target phosphate with Phos-tag™. The phenolic or amine matrices (see below) are recommended for phosphorylated peptides and phospholipids (e.g., lysophosphatidic acid). The matrix solution (10~ 40 mg/mL) in an appropriate organic solvent should be prepared prior to use.

**Note:** Do not use acidic matrices such as cinnamic acid derivatives.



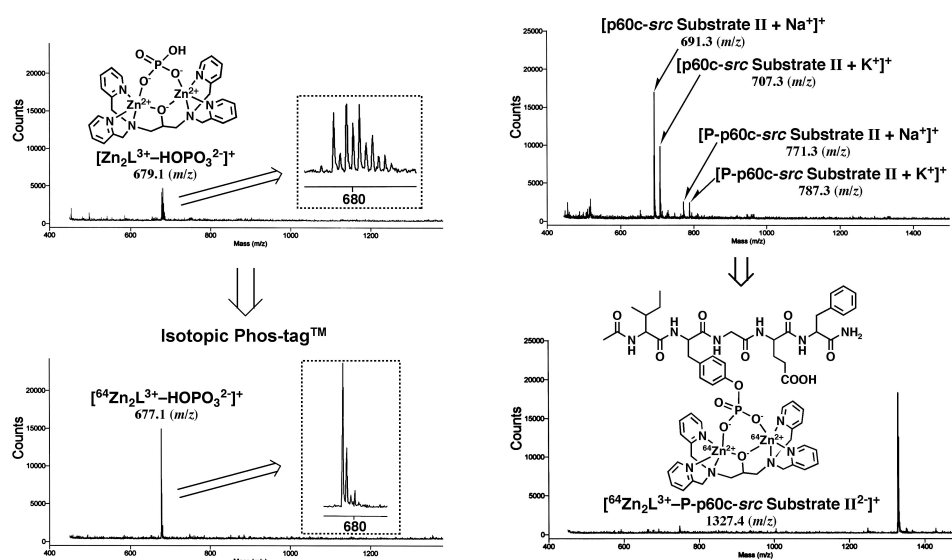
## Phos-tag™ Solution

The stock solution of 1.0 mM Phos-tag™ MS-101 (acetate-bound form) is prepared using deionized water. Do not adjust pH with acid or base. The solution pH is around 6. Store at 4 °C.

## Sample Preparation on the Sample Plate

Mix sample solution, matrix solution and Phos-tag™ solution directly on the sample plate (the total volume ca. 1  $\mu\text{L}$ ). The volume ratio of the solutions is optimized for each sample in a similar manner as reported for the general MALDI-TOF MS analysis. The solvent of the mixture is evaporated under an appropriate condition. Load the sample plate in the mass spectrometer.

Mass spectra of Phos-tag complexes with inorganic phosphate and phosphopeptide



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