Interchim Innovations

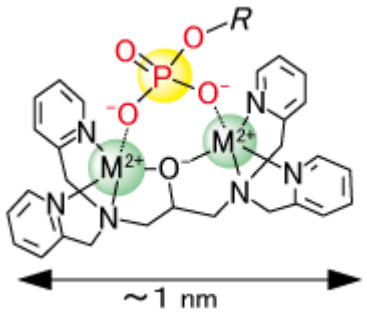
Interbiotech - BioScience Innovations

H10E

Phosphorylation study – separation & detection by Phos-Tag technology

Phos-tag™ binds specifically phosphorylated ions and allows specific separation of phosphorylated proteins by electrophoresis (Phos-tag™ Acrylamide), enrichment, separation and purification (Phos-tag™ Agarose), as well as for the detection using western blot (Phos-tag™ Biotin) and MALDI-TOF/MS (Phos-tag™ Mass Analytical Kit). It is a valuable method for determining the phosphorylation status of proteins (i.e., phosphoproteomics), with respect to the evaluation of diverse biological and pathological processes.

Basic Structure of Phos-tag™】



M^{2+} : Zinc ion or manganese ion

Features:

- **Selective** binding of phosphomonoester dianion (PO_4^{2-})
  Recognition of all phosphorylated forms of Ser / Thr / Tyr
- **Stable complex** under physiological conditions (pH 5 to 8).
  Generally dissociates at a pH of 3 to 4 – usefull for capture methods -.
- Radioisotope labeling is not required
- Downstream applications followed by mass analysis, etc. are applicable.
- Capture and detection applications

Product	Purpose of Use
Phos-tag™ Acrylamide	**Separation :** Separation is possible by SDS-PAGE depending on the degree of phosphorylation.
SuperSep Phos-tag™	**Separation :** Ready-to-Use Precast gel containing 50 µM Phos-tag™ Acrylamide
Phos-tag™ Agarose	**Purification :** Phosphorylated proteins are purified by column chromatography
Phos-tag™ Biotin	**Detection :** A substitute for the anti-phospho antibody used in western blot.
Phos-tag™ Mass Analytical Kit	**Analysis :** This is used in MALDI-TOF/MS analysis to improve the detection sensitivity of phosphorylated molecules.

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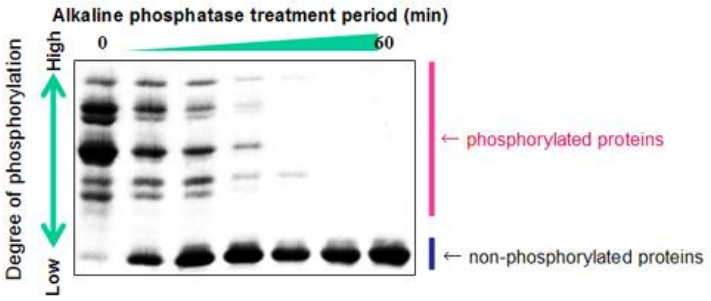


■ Phos-Tag reagents for electrophoresis analysis

Phos-tag Acrylamide FL8630, 2mg FL8631, 10mg #FL863
Molecular weight: 594.7 FL8640, 0.3 mL(0.9 mg) #FL864

Amount of ligand (Form) :Phos-tag/Acrylamide=1/1(Mn^{2+}-unbound ligand) - to be use a ~50µg/ml in gels.
Technical Sheet: FT-FL8630() : Mobility Shift Detection of Phosphorylated Proteins- Phosphate Affinity SDS-PAGE using Acrylamide-Phos-tag™

Technical Notice: NT-FL8631(). Protocols, Applications…

■ Phos-Tag Supports for enrichment, separation, and purification

Phos-tag Agarose 1H6360, 0.5mL FL8641, 3mL
Binding site: 3 ~ 5 µmol Phos-tag
More information: NT-FL8631.pdf#page=26()

Technical Sheet: FT-1H6360() : Enrichment of Phosphorylated Proteins from Cell Lysate-Phosphate Affinity Chromatography using Phos-tag Agarose

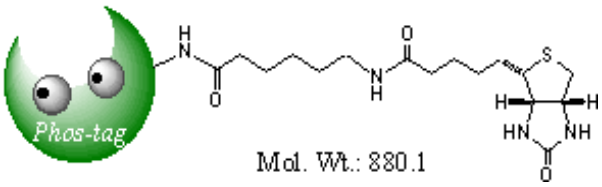
■ Phos-Tag Kit for mass spectrometry analysis (Isotope-labeled Phostag)

Phos-tag Mass Analytical Kit		987890, 1 kit
Kit contents:	BZ6810, MS-101L, 5 mg	MW: 581.4
	BZ6820, MS-101H, 5 mg	MW: 589.4
	BZ6830, MS-101N, 10 mg	MW: 584.3

Technical notice: FT-987890() : MALDI-TOF Mass Analysis of Phosphorylated Molecules- Detection of Phosphate-binding Isotopic Zn2+–Phos-tag™-

■ Phos-Tag Reagents for W-blotting, surface plasmon resonance, and separation

Phos-tag™-Biotin	BTL-104(301-93531)	#FL823
MW : 767		
Phos-tag™-LC-Biotin	BTL-105(308-93541)	#FL824
MW : 880.1		
Phos-tag™-PEO_{12}-Biotin	BTL-111(308-97201)	#0B884
MW :1367		



Amount of ligand (Form): Phos-tag Biotin = 1/1 (Zn2+-unbound ligand)
More information: NT-FL8631.pdf#page=25()

PhosTag-Biotin can be used as an alternative to an antiphospho antibody, notably when using a anti-serine/threonine phospho antibody is difficult.
It useful also in a variety of immunoassay, as well as for SPR experiments, and separation/immunoprecipitation

Phos-tag™ Tips －Sample extraction of phosphorylated proteins by affinity chromatography

Phos-tag™ Tip	8Tips	#AG2-103(387-07321)
3 ~ 5 µmol Phos-tag/mL-gel		#B4VPN
10µL-gel/Tip		

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Applications:

Phos-Tag is a unique tool for the study of the phosphorylation and dephosphorylation of proteins processes, relevant to information transmission in living bodies and many life functions - a major theme of post-genome research and in the development of new drugs - :

1: Nuclear magnetic resonance (NMR)
2: Mass spectrometry (MS)
3: Electrophoresis
4: Enrichment, separation, and purification
5: Deactivators
6: Enzyme inhibitors
7: Surface plasmon resonance (SPR)
8: Western blotting

The phosphate group of a phosphorylated compound can be capped to detect an unknown donor by nuclear magnetic resonance or mass spectrometry measurements of the spin difference or mass difference with respect to a reference sample. Chromatography can be used to detect the change in mass that is caused by Phos-tag binding to the analyzed compound, and electrophoresis can be used due to the electrical change caused by the addition of Phos-tag's charge. Phos-tag can be bounded to a plate, resin, beads, fibers, etc. for capture of a donor and separation of phosphorylated compounds from non-phosphorylated compounds. By selection of the plate, resin, fibers, etc. to which Phos-tag is bound, separation according to the number of phosphate groups is enabled.

Technical tip ● principle

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) acts as a selective phosphate-binding tag molecule, Phos-tag™ in an aqueous solution at a neutral pH (e.g., Kd = 25 nM for phenyl phosphate dianion, Ph-OPO32-).
M2+: Zinc ion or Manganese ion

Under neutral pH conditions (physiological conditions), Phos-tag binds with an anionic substituent, Selectivity of binding of a phosphomonoester dianion (phosphate ion^{2-}) is much higher than that of other anions. with a. Phos-tag can take the place of conventional enzyme immunoassay and radioactive isotope methods as an agent for capturing substances with an anionic substituent, especially a phosphomonoester dianion. Unstable phosphorylated compounds, which were difficult to measure up until now due to instability, can also be stabilized.

● advantage of PhosTag technology

Phos-tag technology solves issues, and overcomes conventional enzyme immunoassay and radioactive isotope methods, as a technology for capturing substances with an anionic substituent, especially a phosphomonoester dianion, as well as stabilizing unstable phosphorylated compounds, which were difficult to measure up until now due to instability.

Conventional analytical methods suffered from many limitations:
- Enzyme immunoassay method
  The enzyme immunoassay method makes use of the specific binding of an antibody with a target substance, thus requires the preparation of an antibody unique to the target substance as well as acquisition and purification of the target substance. This takes time and an animal is used. Furthermore,
  An antibody cannot be prepared for a phosphorylated part of a molecular structure of a few kDa or less.
-· Radioactive isotope method
  Because it use a radioactive isotope ^{32}P, the method is troublesome technically (protection from radiation, radioioactivity reader) and for safety and regulation issues (control of waste liquids)

Related products/documents

Products HighLights Overview, including:
- Crosslinking tools – PEO/PEG biotinylation agents and AmineControlled conjugation kit
- FluoProbes labeling agents
- Dialysis and Desalting tools – CelluSep tubings, SpectraPor tubings, GebaFlex, FloatALyser, SlideALyser,...

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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