Phos-tag application data

Quantitative analysis of phosphorylateion by *in vitro* Abl kinase assay
～ Mn^{2+}-Phos-tag SDS-PAGE with Laemmli system ～

● SAMPLE INFORMATION

		MW (kDa)
Protein	Abltide	-
Protein status	recombinant (Abltide-GST)	28

● ELECTROPHORESIS CONDITION

Gel	12.5% polyacrylamide
Phos-tag conc.	100μM Mn^{2+} - Phos-tag
Metal complex	

Visualization	CBB stain / immunoblotting
Antibody	anti-pTyr

● ASSAY FLOW

1 Abitide phosphorylation with Abl
2 Phos-tag electrophoresis
3 Quantification by CBB stain and densitometry or immunoblotting

● RESULT

・Mn^{2+}-Phos-tag SDS-PAGE has enabled the simultaneouse determination of phospholylated and corresponding dephosphorylated protein in gel.
・The Mn^{2+}-Phos-tag SDS-PAGE can identify the time course ratio of phosphorylated and dephosphorylated proteins in gel.

● NOTE

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● REFERENCE

Phosphate-binding tag, a new tool to visualize phosphorylated proteins. Kinoshita E, Kinoshita-Kikuta E, Takiyama K, Koike T. : *Mol. Cell. Proteomics* , **5**, 749 (2006)

key words : Mn^{2+}-Phos-tag, Laemmli, Abl, tyrosine kinase