Phos-tag application data

Phosphate affinity 2-D DIGE analysis of phosphorylation status of cellular proteins
～ Comparison of Bis-Tris-HCl gel system and Tris-AcOH gel system ～

● SAMPLE INFORMATION

		MW (kDa)
Protein	Mixture of lysate (HeLa cell)	-
Protein status	labeling Cy5、labeling Cy3	200以下

● ELECTROPHORESIS CONDITION

Gel	1D: 8～12% polyacrylamide (without Phos-tag) 2D: 8.0, 6.5, 5.5% polyacryamide (Bis-Tris-HCl gel)
Phos-tag conc.	50μM
Metal complex	Zn^{2+}

Visualization	fluorescence (2D-DIGE)
Antibody	-

● ASSAY FLOW

1 Preparation of cell lysate and calyculin treatment
2 Cy5 labeling of calyculin A treated cell lysate, Cy3 labeling of no treated cell lysate
3 Normal SDS-PAGE as the first dimention
4 Phos-tag SDS-PAGE as the second dimention
5 Fluorescence analysis and mass spectrometry analysis

● RESULT

・Zn^{2+}-Phos-tag SDS gels in Bis-tris-HCl buffer showed ditaled phosphorylation status, than Tris-AcOH buffer.
・Tris-AcOH buffer showed dominat result at higher molecural proteins than 200kD.

● NOTE

Another data of simultaneous analysis : 006-2

● REFERENCE

Phos-tag SDS-PAGE systems for phosphorylation profiling of proteins with a wide range of molecular masses under neutral pH conditions. Kinoshita E, Kinoshita-Kikuta E, Koike T. : *Proteomics* , **12**, 192 (2012)

key words : 2D-DIGE, 2D-PAGE, Zn^{2+}-Phos-tag, Bis-Tris-HCl, Tris-AcOH, HMW, HeLa