Phos-tag application data

Phosphorylation analysis of extracellular signal-regulated kinase (Erk)
～ Comparison of mobility between Bis-Tris-HCl buffer system and Tris-AcOH buffer system (1) ～

● SAMPLE INFORMATION

		MW (kDa)
Protein	Erk1, Erk2 (A431)	~ 200
Protein status	normal	-

● ELECTROPHORESIS CONDITION

Gel	8.0% polyacrylamide (Bis-TrisHCl, Tris-AcOH) 1D: 8.0% polyacrylamide (without Phos-tag) 2D: 8.0% polyacrylamide (Bis-Tris-HCl, Tris-
Phos-tag conc.	25μM
Metal complex	Zn2+

Visualization	immunoblotting
Antibody	anti-Erk1/2

● ASSAY FLOW

1 EGF stimulation of cell lysate
2 Phos-tag electrophoresis
2'-1 Normal SDS-PAGE as the first dimention
2'-2 Phos-tag SDS-PAGE as the second dimention
3 Immunoblotting

● RESULT

・Comparison by Bis-Tris-HCl gel and Tris-AcOH showed same shifted bands.
・2D-PAGE proved overlapping of Erk1 and Erk2 in each shifted bands.

● NOTE

Another data of simultaneous analysis : 007-2 007-3

● 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 : Bis-Tris-HCl, Tris-AcOH, Zn^{2+}-Phos-tag, 2D-PAGE, Erk, Erk1, Erk2, A431, HMW