

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 (3)

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● SAMPLE INFORMATION

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

● ELECTROPHORESIS CONDITION

Gel	8.0% polyacrylamide (Bis-TrisHCl, Tris-AcOH)
Phos-tag conc.	25μM
Metal complex	Zn ²⁺

Visualization	immunoblotting
Antibody	anti-pT202, anti-pY204 anti-Erk1/2

● ASSAY FLOW

- 1 EGF stimulation of cell lysate
- 1' Calyculin A, prevanadate or PD98059 stimulation of cell lysate
- 2 Phos-tag electrophoresis
- 3 Immunoblotting

● RESULT

·This method analysed continuous proceeded reaction of kinase and phosphatase.

● NOTE

Another data of simultaneous analysis : 007-1 007-2

● 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 : Bis-Tris-HCl, Tris-AcOH, Zn²⁺-Phos-tag, Erk, Erk1, Erk2, A431, HMW