Purification of proteins binding Bisindolylmaleimide X

This study is the collaboration with a pharmaceutical company.

Bisindolylmaleimide is a compound with a structure in which two indole rings are bonded to a maleimide group, and Bisindolylmaleimide X (BisX) with this skeleton acts as a protein kinase (PKC) inhibitor. In this experiment, Bisindolylmaleimide X hydrochloride was immobilized on FG beads® and bound proteins were purified from cell extracts.

Method 1 Immobilization of Bisindolylmaleimide X hydrochloride (based on Protocol 014)

NHS beads 20 mg (5 mg×4)

↓

Immobilization reaction

Concentration (mM)	0	0.1	0.3	1
NHS beads (mg)	5	5	5	5
2mM BisX・HCl (uL)	0	50	150	500
4mM Et_3N (uL)	0	50	150	500
DMF (uL)	1000	900	700	0
Total (uL)	1000	1000	1000	1000

70 minutes, room temperature

↓

Masking　1 M aminoethanol, 1000 µl

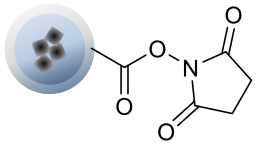
2 hours, room temperature

↓

Washing　50% MeOH 1000 µL, 3 times

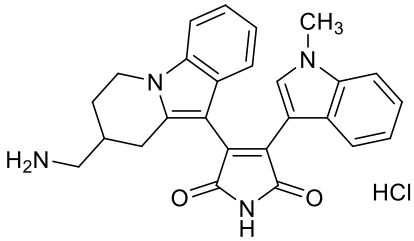
↓

BisX immobilized beads （0 mM, 0.1 mM, 0.3 mM, 1 mM） 5 mg each



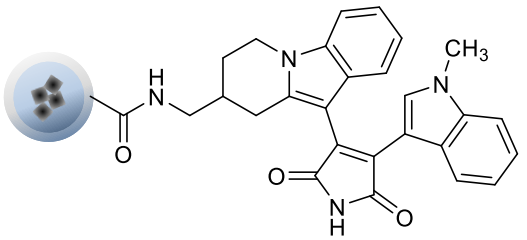
NHS beads
(TAS8848N1141)

↓



Bisindolylmaleimide X
hydrochloride

↓



BisX immobilized beads

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Method 2 Affinity purification with Bisindolylmaleimide X immobilized beads (based on Protocol 001)

BisX immobilized beads（0 mM, 0.1 mM, 0.3 mM, 1 mM） 0.5 mg each

↓

Washing — 100mM KCl buffer 200μL, 3 times

↓

Binding reaction — Cell extracts 0.2 mg/mL, 1000μL

↓ **4 hours, 4℃**

Washing — 100mM KCl buffer 200μL, 3 times

↓

Salt elution — 1M KCl buffer※2

↓

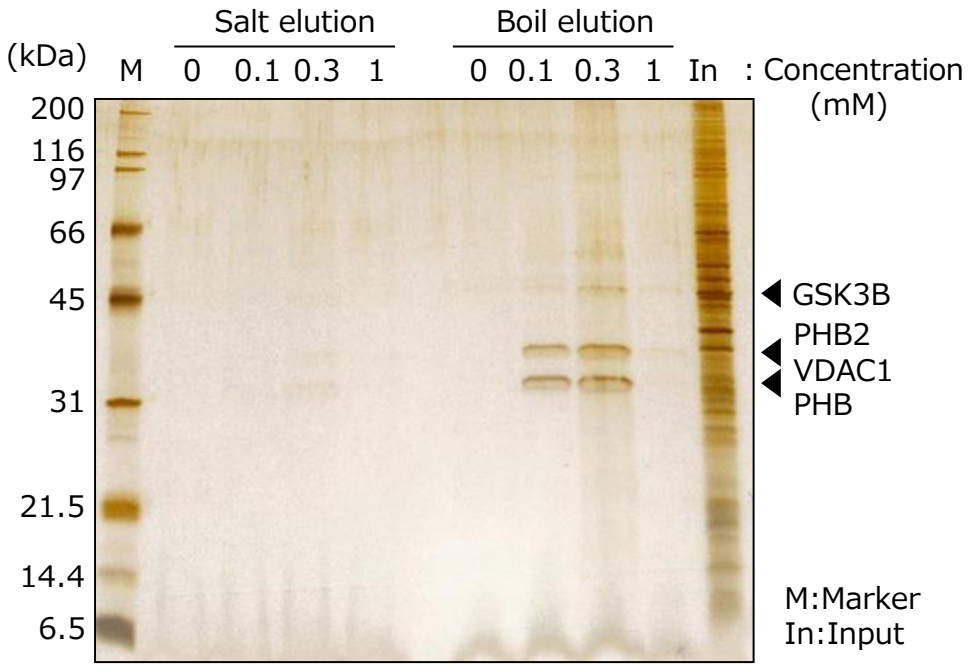
Boil elution — SDS sample buffer※3

↓

SDS-PAGE

↓

Silver stain



BisX binding proteins (GSK3β, PHB2, VDAC1, PHB) were purified with high purity by affinity purification with BisX immobilized beads.

※1 : 100 mM KCl buffer : 20 mM HEPES-NaOH(pH7.9), 100 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM EDTA, 10% glycerol, 0.1% NP-40, 1 mM DTT, 0.2 mM PMSF

※2 : 1 M KCl buffer : 20 mM HEPES-NaOH(pH7.9), 1 M KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM EDTA, 10% glycerol, 0.1% NP-40, 1 mM DTT, 0.2 mM PMSF

※3 : SDS sample buffer : 62.5 mM Tris-HCl(pH6.8), 0.005% BPB, 2% SDS, 10% glycerol, 5% 2-Mercaptoehanol