

Purification of proteins binding FK506(Tacrolimus)

FK506 is an immunosuppressive drug collected from soil bacteria by Fujisawa Pharmaceutical Co., Ltd. (currently Astellas Pharma Inc.) and clinically developed, and is known to be 100 times more effective than the same type of cyclosporin A. In this experiment, FK506 was immobilized on FG beads, and FKBP12, which is known as a binding protein of FK506, was purified from cell extracts.

Method 1 Immobilization of FK506 (Protocol 016)

COOH beads 4 mg (1 mg ×4 conditions)

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Immobilization

Concentration of FK506 (mM)	0	3	10	30
COOH beads (mg)	1	1	1	1
DMF (μl)	200	176	126	0
100 mM i-Pr2NEt (μl)	0	10	30	90
100 mM TFFH (μl)	0	4	12	36
100 mM FK506 (μl)	0	6	20	60
10 mM Oxyma (μl)	0	4	12	36
Total (uL)	200	200	200	200

over night, room temperature

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Activation

DMF 160 μl, 1M HOSu 40 μl, EDC · HCl 7.7 mg

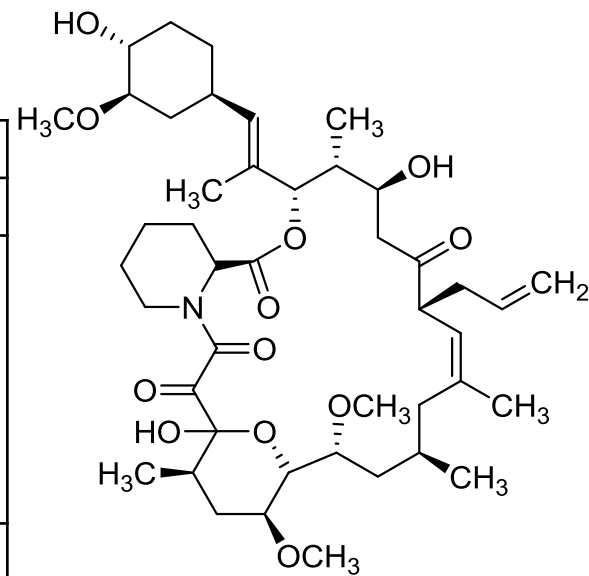
2 hours, room temperature

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Masking(Blocking)

1 M aminoethanol 200 μl

2 hours, room temperature

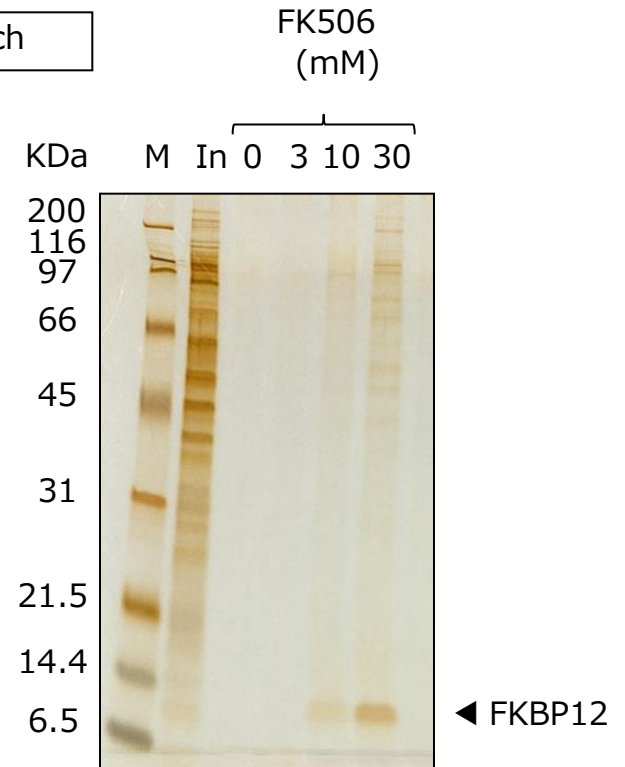
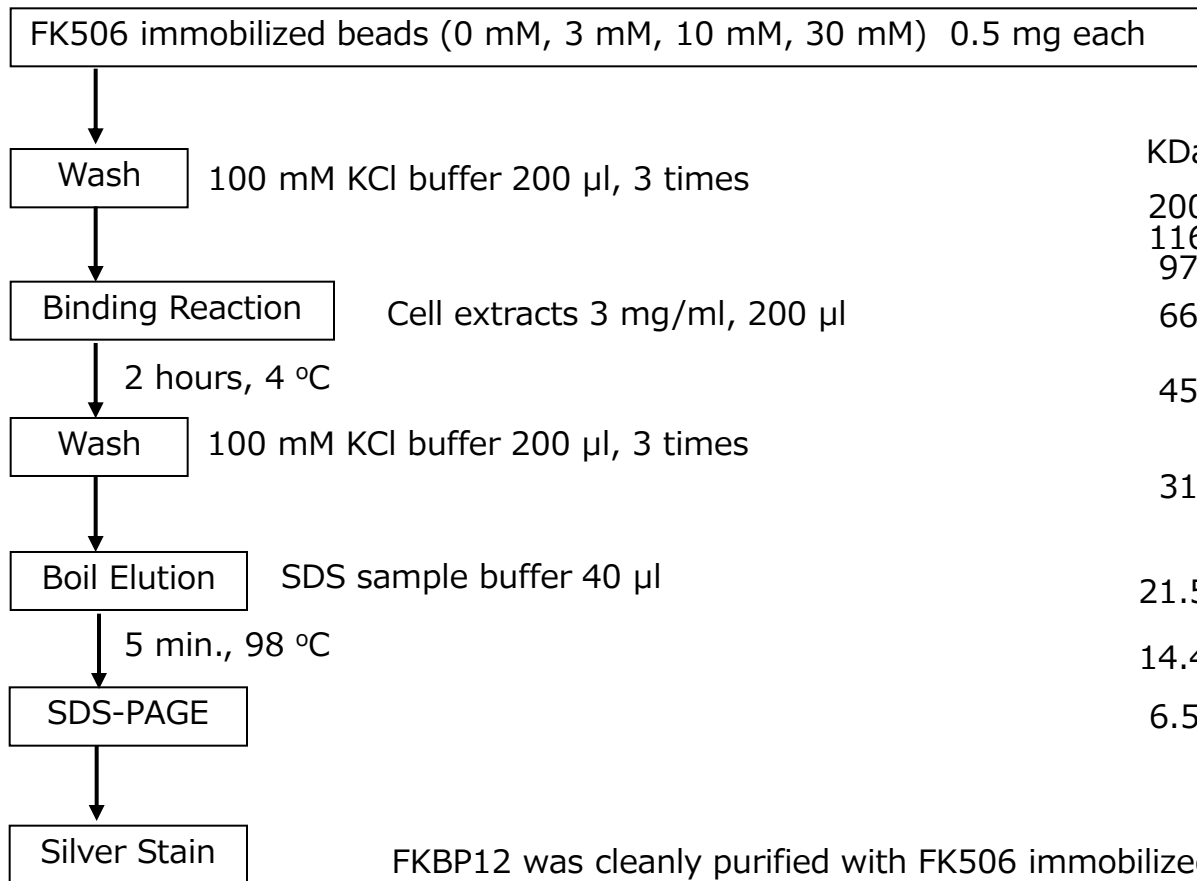
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FK506 immobilized beads (0 mM, 3 mM, 10 mM, 30 mM) 1 mg each



FK506
(Tacrolimus)

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Method 2 Affinity purification with FK506 immobilized beads (Protocol 001)



FKBP12 was cleanly purified with FK506 immobilized beads.
The amount of FKBP12 recovered increased in proportion to the amount of FK506 immobilized on the beads.
FKBP12 was identified by mass spectrometry.