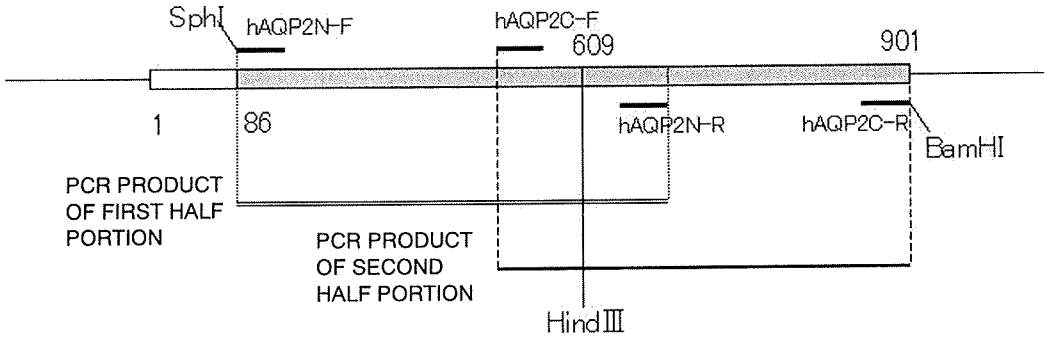
[Fig. 1]

hAQP2 / FIRST HALF PORTION PRIMER

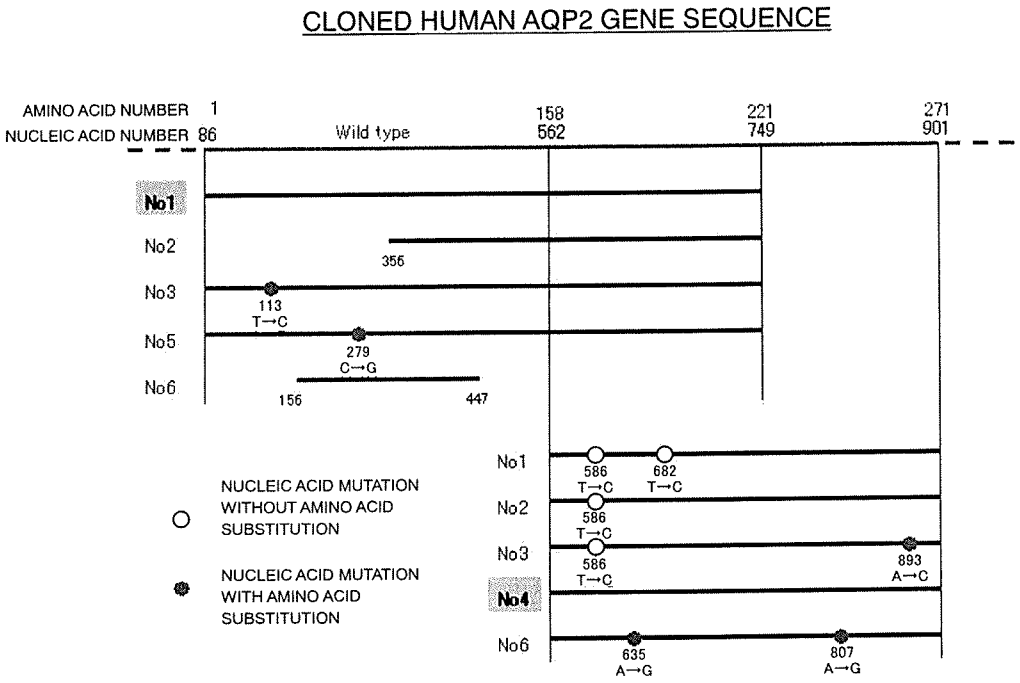
hAQP2N-F; 5'-GG **GCA TGC** ATG TGG GAG CTC CGC TCC ATA G-3'
SphI 86 107
hAQP2N-R; 5'-CGT AGT TGT AGA GGA GGG AGC C-3'
749 728

hAQP2 / SECOND HALF PORTION PRIMER

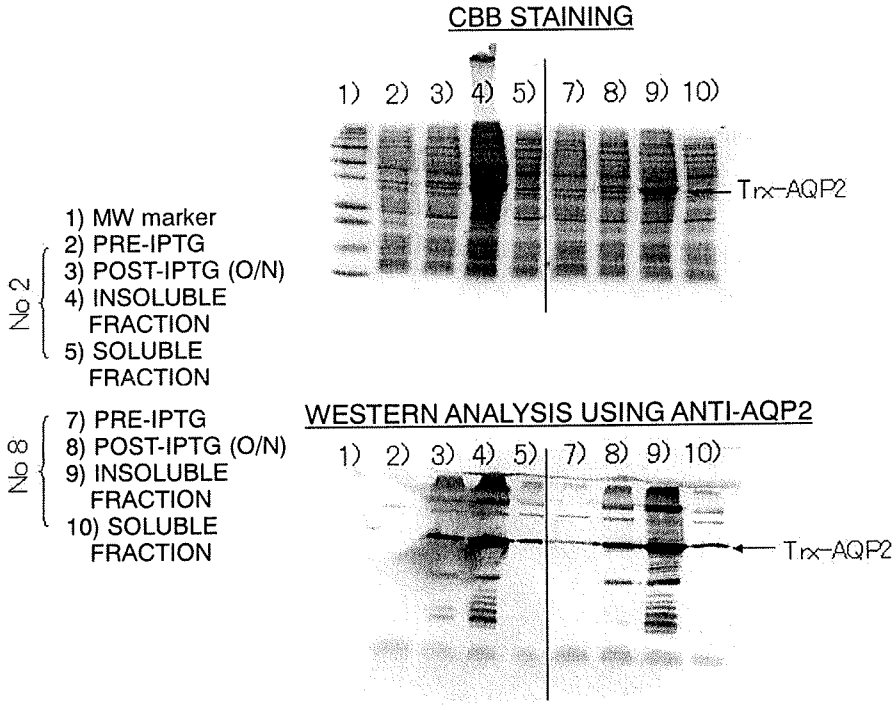
hAQP2C-F; 5'-CCC TGC TCT CTC CAT AGG CTT C-3'
562 583
hAQP2C-R; 5'-CC **AAG CTT** TCA GGC CTT GGT ACC CCG TGG-3'
BamHI 901 881



[Fig. 2]

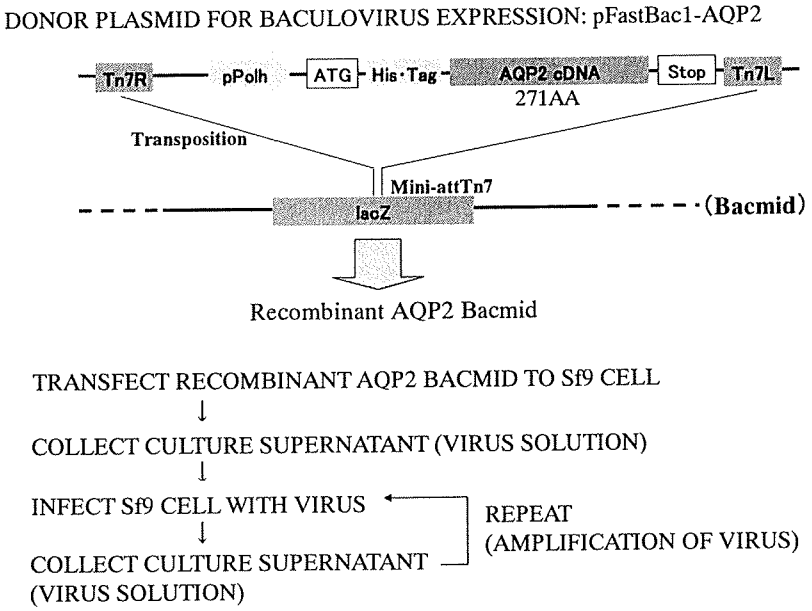

CLONED HUMAN AQP2 GENE SEQUENCE
AMINO ACID NUMBER 1
NUCLEIC ACID NUMBER 86
Wild type
158
562
221
749
271
901
No1
No2
356
No3
113
T→C
No5
279
C→G
No6
156
447
NUCLEIC ACID MUTATION WITHOUT AMINO ACID SUBSTITUTION
NUCLEIC ACID MUTATION WITH AMINO ACID SUBSTITUTION
No1
586
T→C
682
T→C
No2
586
T→C
No3
586
T→C
893
A→C
No4
No6
635
A→G
807
A→G

[Fig. 3]

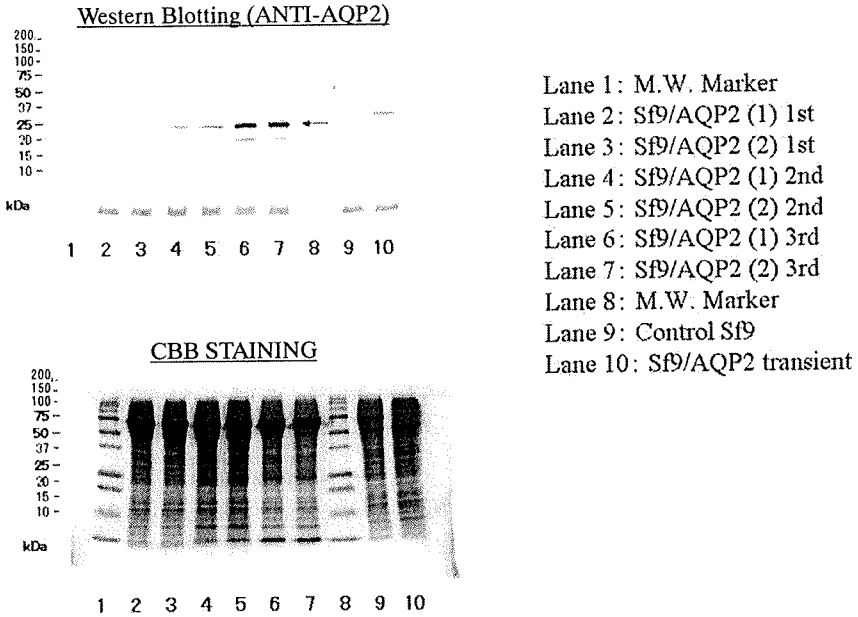

CBB STAINING
1) 2) 3) 4) 5) 7) 8) 9) 10)
Trx-AQP2
1) MW marker
No 2
2) PRE-IPTG
3) POST-IPTG (O/N)
4) INSOLUBLE FRACTION
5) SOLUBLE FRACTION
No 8
7) PRE-IPTG
8) POST-IPTG (O/N)
9) INSOLUBLE FRACTION
10) SOLUBLE FRACTION
WESTERN ANALYSIS USING ANTI-AQP2
1) 2) 3) 4) 5) 7) 8) 9) 10)
Trx-AQP2

[Fig. 4]

(1)



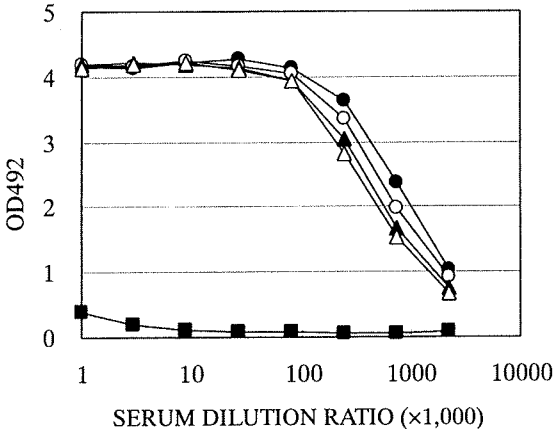
(2)



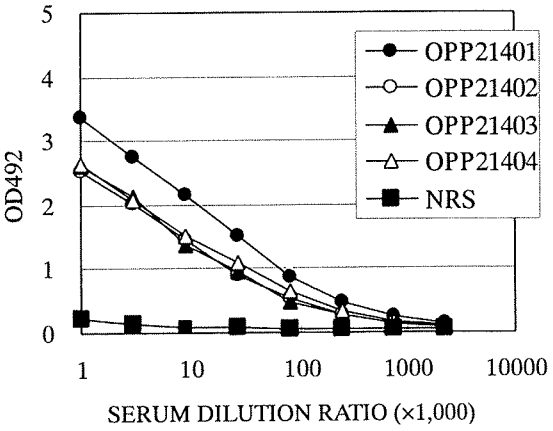
Lane 1: M.W. Marker
Lane 2: Sf9/AQP2 (1) 1st
Lane 3: Sf9/AQP2 (2) 1st
Lane 4: Sf9/AQP2 (1) 2nd
Lane 5: Sf9/AQP2 (2) 2nd
Lane 6: Sf9/AQP2 (1) 3rd
Lane 7: Sf9/AQP2 (2) 3rd
Lane 8: M.W. Marker
Lane 9: Control Sf9
Lane 10: Sf9/AQP2 transient

[Fig. 5]

(A)

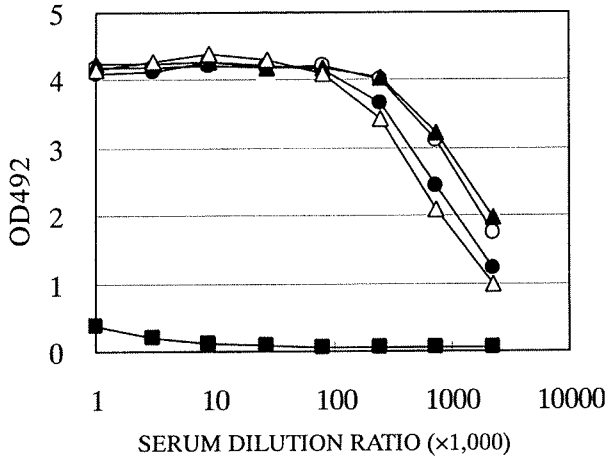


(B)

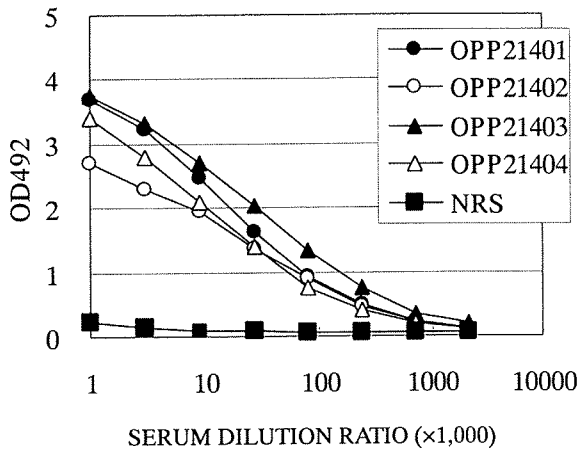


[Fig. 6]

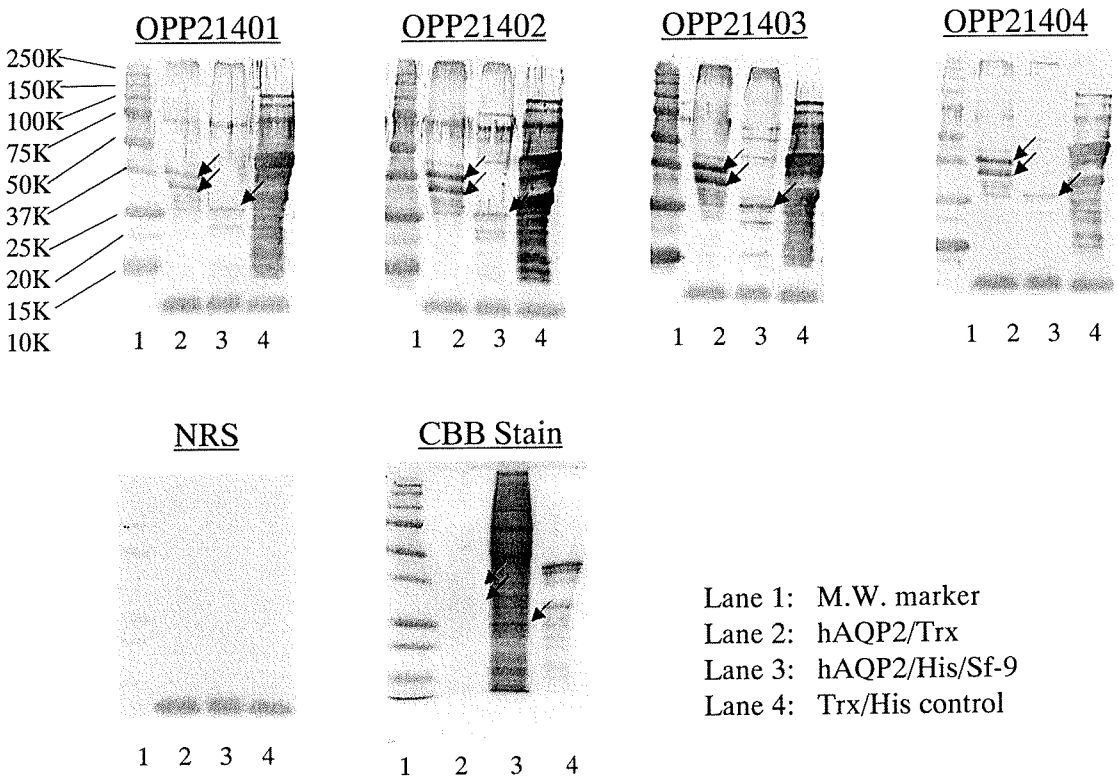
(A)



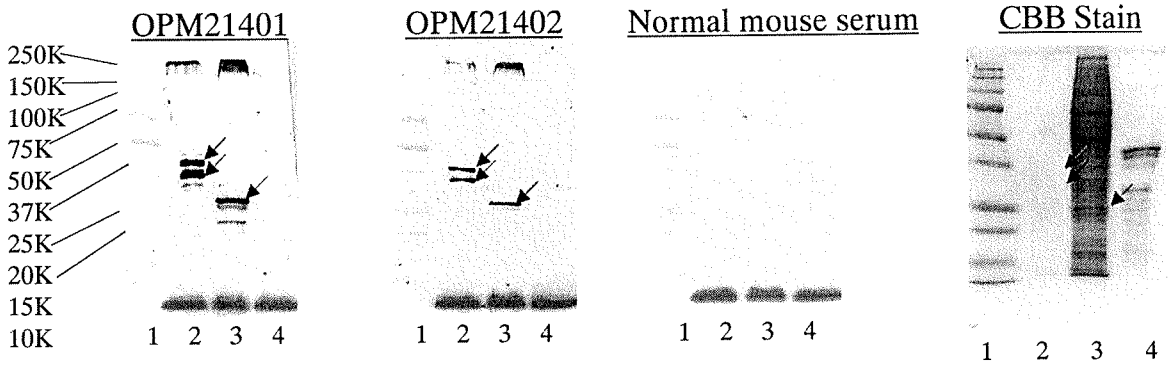
(B)



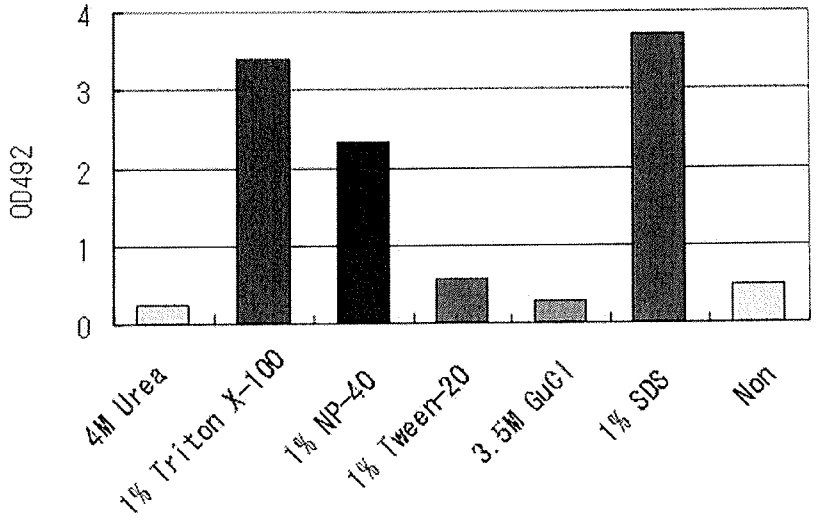
[Fig. 7]


OPP21401
OPP21402
OPP21403
OPP21404
250K
150K
100K
75K
50K
37K
25K
20K
15K
10K
1 2 3 4
NRS
CBB Stain
Lane 1: M.W. marker
Lane 2: hAQP2/Trx
Lane 3: hAQP2/His/Sf-9
Lane 4: Trx/His control

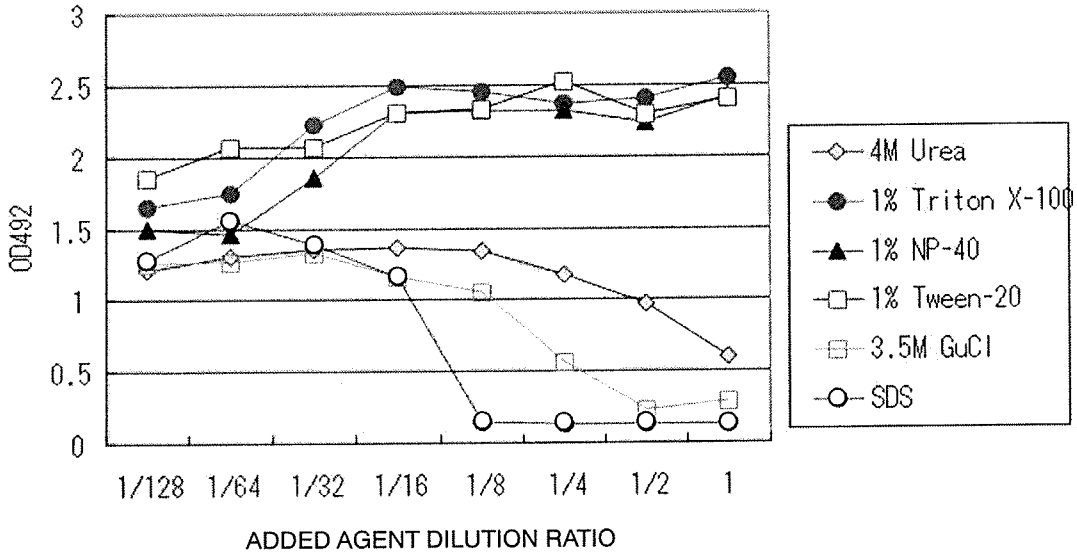
[Fig. 8]


OPM21401
OPM21402
Normal mouse serum
CBB Stain
250K
150K
100K
75K
50K
37K
25K
20K
15K
10K
1 2 3 4
Lane 1: M.W. marker
Lane 2: hAQP2/Trx
Lane 3: hAQP2/His/Sf-9
Lane 4: Trx/His control

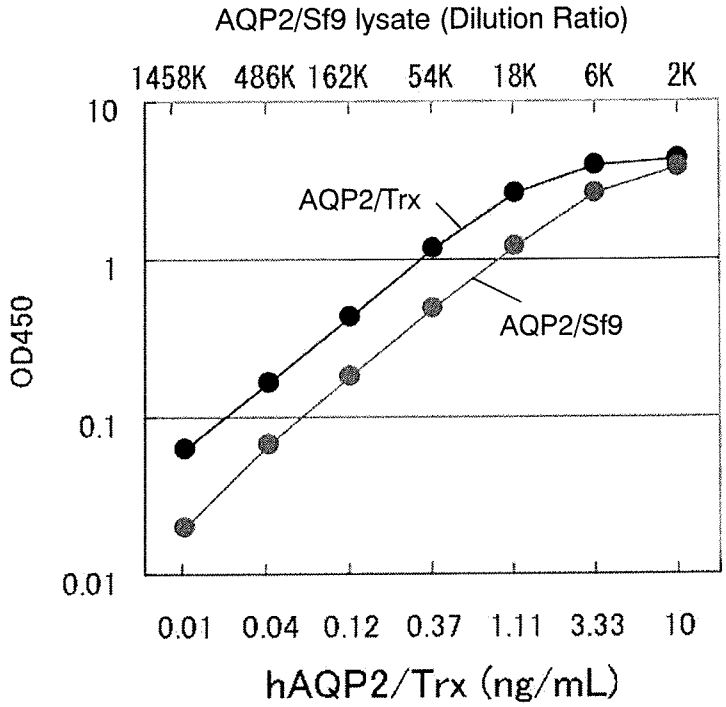
[Fig. 9]


OD492
4
3
2
1
0
4M Urea
1% Triton X-100
1% NP-40
1% Tween-20
3.5M GuCl
1% SDS
Non

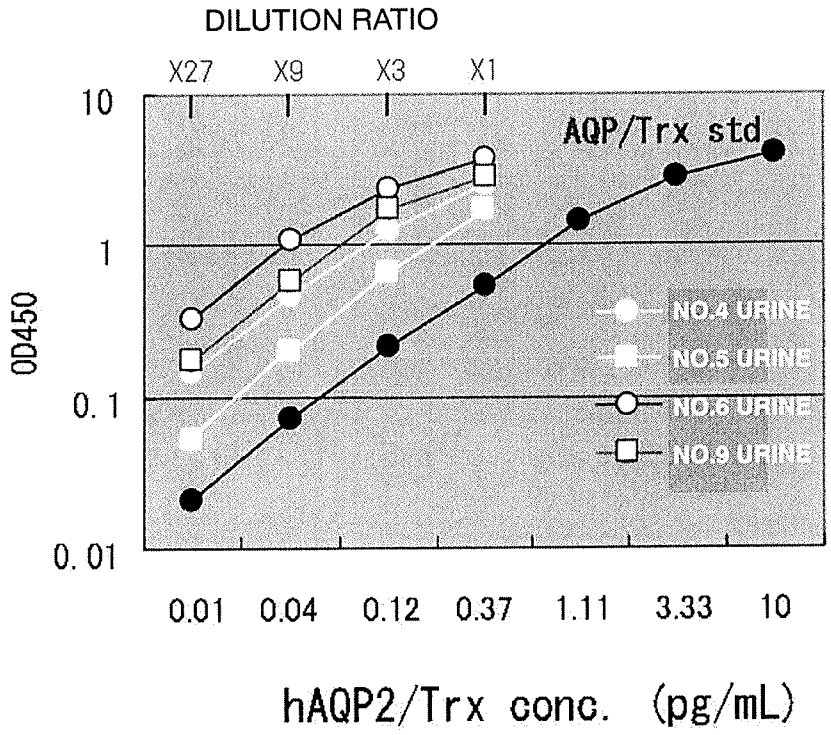
[Fig. 10]


OD492
3
2.5
2
1.5
1
0.5
0
1/128
1/64
1/32
1/16
1/8
1/4
1/2
1
ADDED AGENT DILUTION RATIO
4M Urea
1% Triton X-100
1% NP-40
1% Tween-20
3.5M GuCl
SDS

[Fig. 11]

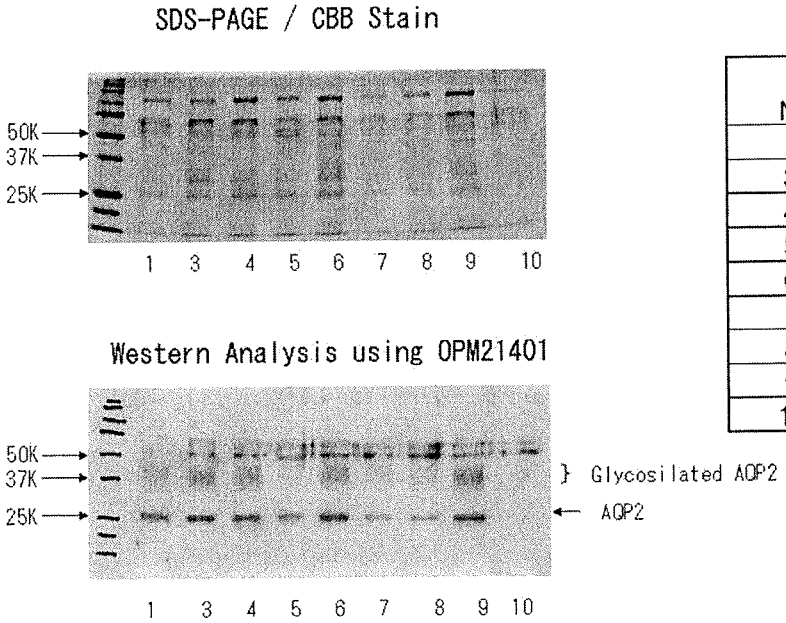

AQP2/Sf9 lysate (Dilution Ratio)
1458K 486K 162K 54K 18K 6K 2K
10
1
0.1
0.01
OD450
AQP2/Trx
AQP2/Sf9
0.01 0.04 0.12 0.37 1.11 3.33 10
hAQP2/Trx (ng/mL)

[Fig. 12]


DILUTION RATIO
X27 X9 X3 X1
10
1
0.1
0.01
OD450
AQP/Trx std
NO.4 URINE
NO.5 URINE
NO.6 URINE
NO.9 URINE
0.01 0.04 0.12 0.37 1.11 3.33 10
hAQP2/Trx conc. (pg/mL)

[Fig.13]

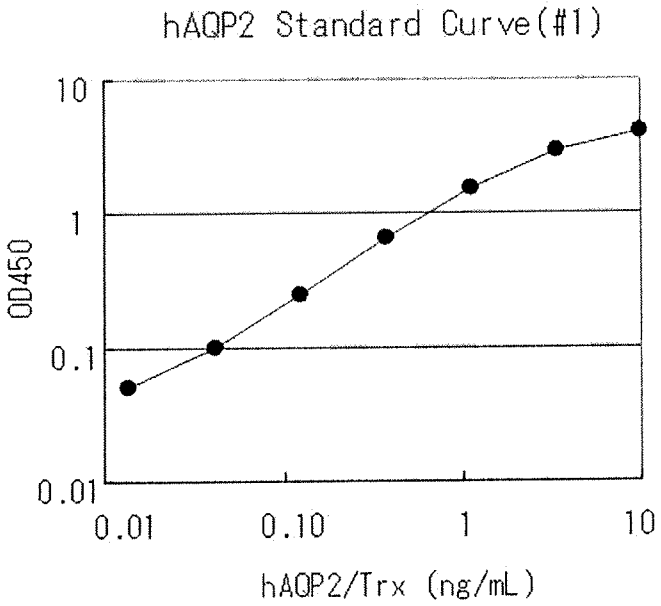
(A)



(B)

No	AQP2 (ng/mL)
1	0.95
3	3.28
4	4.04
5	1.12
6	8.84
7	1.06
8	0.93
9	4.88
10	#

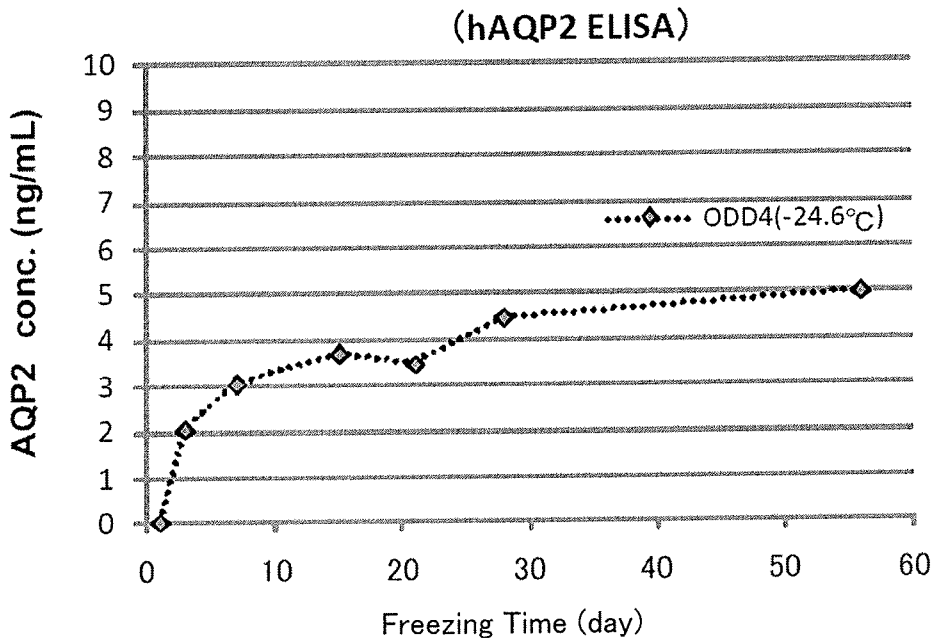
[Fig.14]



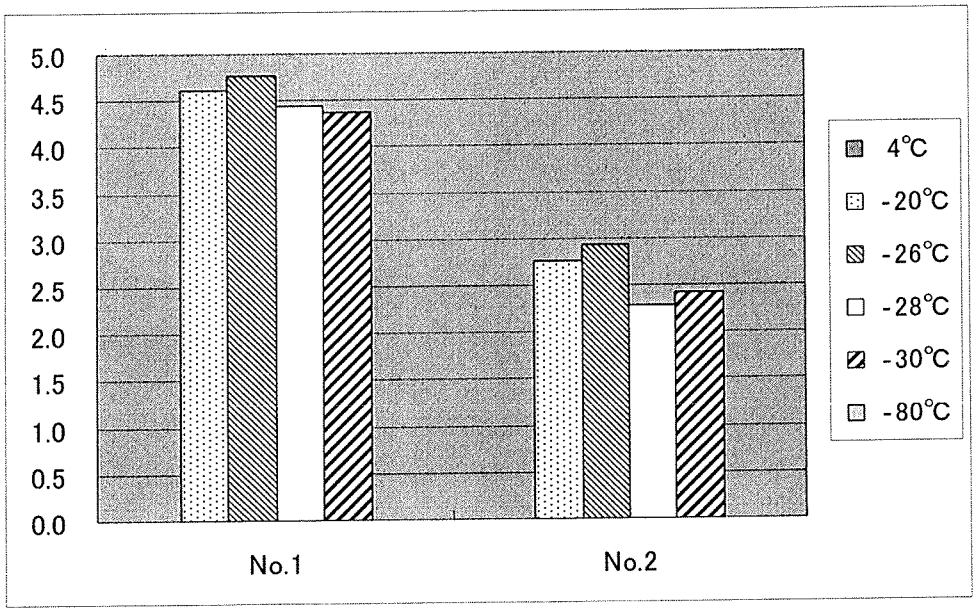
[Fig. 15]

	1st					2nd		3rd		4th		5th
	PRESERVATION TEMPERATURE											
A	A: 37℃	5>		PRESERVED AT 4℃	A	1.0	PRESERVED AT 4℃	8.0	PRESERVED AT 4℃	6.5	PRESERVED AT 4℃	10.4
B	B: 23℃	5>			B	0.7		1.1		1.2		2.5
C	C: 4℃	5>			C	0.7	→	1.4	→	0.0	→	1.4
D	D: -30℃	100	→		D	100		100		100		100
E	E: -80℃	5>			E	4.3		5.6		3.6		5.5
				PRESERVED AT -30℃	A	103.5	PRESERVED AT -30℃	100.8	PRESERVED AT -30℃	104.3	PRESERVED AT -30℃	80.6
					B	88.6		95.1		119.0		94.6
			→		C	78.2	→	91.7	→	95.3	→	82.8
					D	99.6		103.6		90.3		82.2
					E	72.8		91.7		92.1		85.7
				PRESERVED AT -80℃	A	9.4	PRESERVED AT -30℃	100.4	PRESERVED AT -30℃	108.6	PRESERVED AT -30℃	90.9
					B	0.9		97.5		113.8		101.0
			→		C	1.9	→	97.2	→	106.8	→	100.4
					D	90.6		102.3		114.8		104.9
					E	3.9		100.7		103.9		86.0

[Fig. 16]


(hAQP2 ELISA)
AQP2 conc. (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
ODD4(-24.6℃)
0
10
20
30
40
50
60
Freezing Time (day)

[Fig. 17]


5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No.1
No.2
4℃
-20℃
-26℃
-28℃
-30℃
-80℃

SEQUENCE LISTING

<110> OTSUKA PHARMACEUTICAL CO., LTD.
<110> NATIONAL UNIVERSITY CORPORATION TOKYO MEDICAL AND DENTAL UNIVERSITY

<120> Method for pre-treating of biological sample cintaining a protein

<130> P12-114

<150> JP 2011-176272
<151> 2011-08-11

<160> 5

<170> PatentIn version 3.4

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1 5 10 15

Phe Leu Ala Thr Leu Leu Phe Val Phe Phe Gly Leu Gly Ser Ala Leu
20 25 30

Asn Trp Pro Gln Ala Leu Pro Ser Val Leu Gln Ile Ala Met Ala Phe
35 40 45

Gly Leu Gly Ile Gly Thr Leu Val Gln Ala Leu Gly His Ile Ser Gly
50 55 60

Ala His Ile Asn Pro Ala Val Thr Val Ala Cys Leu Val Gly Cys His
65 70 75 80

Val Ser Val Leu Arg Ala Ala Phe Tyr Val Ala Ala Gln Leu Leu Gly
85 90 95

Ala Val Ala Gly Ala Ala Leu Leu His Glu Ile Thr Pro Ala Asp Ile
100 105 110

Arg Gly Asp Leu Ala Val Asn Ala Leu Ser Asn Ser Thr Thr Ala Gly
115 120 125

Gln Ala Val Thr Val Glu Leu Phe Leu Thr Leu Gln Leu Val Leu Cys
130 135 140

Ile Phe Ala Ser Thr Asp Glu Arg Arg Gly Glu Asn Pro Gly Thr Pro
145 150 155 160

Ala Leu Ser Ile Gly Phe Ser Val Ala Leu Gly His Leu Leu Gly Ile
165 170 175

His Tyr Thr Gly Cys Ser Met Asn Pro Ala Cys Ser Leu Ala Pro Ala
180 185 190

Val Val Thr Gly Lys Phe Asp Asp His Trp Val Phe Trp Ile Gly Pro
195 200 205

Leu Val Gly Ala Ile Leu Gly Ser Leu Leu Tyr Asn Tyr Val Leu Phe
210 215 220

Pro Pro Ala Lys Ser Leu Ser Glu Arg Leu Ala Val Leu Lys Gly Leu
225 230 235 240

Glu Pro Asp Thr Asp Trp Glu Glu Arg Glu Val Arg Arg Arg Gln Ser
245 250 255

Val Glu Leu His Ser Pro Gln Ser Leu Pro Arg Gly Thr Lys Ala
260 265 270

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<213> Artificial

<220>
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<220>
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<220>
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<400> 4
ccctgctctc tccataggct tc 22

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<213> Artificial

<220>
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