9. APPENDICES

Buffers for isolation of actively oxygen evolving PSII membranes

Buffer A (pH 7.5)	
0.4 M	Sucrose
25 mM	HEPES
1 mM	EDTA
15 mM	NaCl
5 mM	$MgCl_2$
5 mM	$CaCl_2$
2 g/l	BSA (fresh add)
5 mM	Na-ascorbic (fresh add)

Buffer B (pH 6.2)	
25 mM	MES-NaOH
150 mM	NaCl
5 mM	$MgCl_2$

Buffer C (pH 6.2)	(Triton incubation buffer)
1 M	glycinebetaine
25 mM	MES-NaOH
15 mM	NaCl
10 mM	$MgCl_2$
5 mM	$CaCl_2$

Buffer D (pH 6.2)	
1 M	glycinebetaine
25 mM	MES-NaOH
15 mM	NaCl
5 mM	$MgCl_2$
5 mM	$CaCl_2$

Stock solution Triton	
12.5 ml	Triton X-100
37.5 ml	Buffer C without glycinebeatine
5 mM	$CaCl_2$

Stock solution PPBQ (electron acceptor)	
30 mM	In DMSO
	(substance in Ethanol not crystallized)

Buffers for oxygen evolution measurements (OEM)

Buffer OEM (pH 6.3)	
1 M	glycinebetaine
25 mM	MES-NaOH
15 mM	NaCl
5 mM	$MgCl_2$
5 mM	$CaCl_2$

Electron acceptor	
Stock solution FeCy	50 mM in water
Stock solution DCBQ	50 mM in DMSO

Buffers for SDS-Page

Sample buffer (pH 8.0)	
5.2%	LDS
172 mM	Tris-HCl
40 mM	DTT
0.5M	Sucrose
0,01 %	Pyronine

Composition of stock solutions for SDS-PAGE

Stock solutions	Concentrations of constituents
50% w/v Acrylamide stock mixture for resolving gel	49.5% acrylamide, 0.5% bisacrylamide
30% w/v Acrylamide stock mixture for stacking gel	29.2% acrylamide, 0.8% bisacrylamide
Resolving gel buffer (no correction of pH around 9.0)	3 M Tris, 0.65 M MES, 0.5% SDS
Stacking gel buffer (pH is adjusted to 6.8 by HCl)	0.625 M Tris, 0.5% SDS
Reservoir buffer (no correction of pH around 8.5)	25 mM Tris, 192 mM glycine, 0.1% SDS

Gel solution containing 6M Urea

	Resolving gel (18%)	Stacking gel (6%)
50% Acrylamide	3600 µl	————
30% Acrylamide	————	1600 µl
Resolving gel buffer	2000 µl	————
Stacking gel buffer	————	1600 µl
Urea	3.6 g	2.9 g
H_20	1900 µl	2800 µl
10% APS	25 µl	65 µl
TEMED	4.5µl	9 µl

Running Buffer

Running Buffer (pH 8.3) (adjusted at room temperature)	
25 mM	Tris-HCl
192 mM	Glycine
0.1 %	SDS

Staining Buffer (*)

Staining solution	
0.15 %	coomassie brilliant blue R-250
50%	methanol
10%	acetic acid

(*) previously warmed to about 50°

Destaining Buffer (*)

Destaining solution	
25%	methanol
7.5%	acetic acid

(*) previously warmed to about 50°

Buffers for depletions of extrinsic polypeptides

Buffer E (pH 6.0)	
5 mM	$CaCl_2$
10 mM	NaCl
25 mM	MES-NaOH

Buffer F (pH 6.5)	
0.3 M	Sucrose
1.2 M	NaCl
25 mM	MES-NaOH

Buffer G (pH 6.5)	
0.3 M	Sucrose
10 mM	NaCl
25 mM	MES-NaOH

Buffers for Calcium and Manganese depletions

Buffer K (pH 6.5)	
0.4 M	Sucrose
15 mM	NaCl
25 mM	MES-NaOH
Buffer L (pH 6.5)	
0.4 M	Sucrose
15 mM	NaCl
0.25 mM	MES-NaOH

Buffer M (pH 3.0)	
0,4 M	sucrose
20 mM	citrate
15 mM	NaCl
Buffer N (pH 6.5)	
0,4 M	sucrose
50 mM	MES-NaOH
15 mM	NaCl
100 µM	EGTA
Buffer O (pH 6.5)	
25 mM	MES-NaOH
15 mM	NaCl
100 µM	EGTA
Buffer H (pH 9.4)	
20 mM	CHES-NaOH
200 mM	$MgCl_2$

Buffer I (pH 6.5)	
0.4 M	Sucrose
15 mM	NaCl
25 mM	MES-NaOH
5 mM	$MgCl_2$

Buffer J (pH 6.5)	
0.4 M	Sucrose
15 mM	NaCl
25 mM	MES-NaOH
5 mM	$MgCl_2$
1.5 mM	HN_2OH

Buffer DEPLE F (pH 6.5)	
0.4 M	Sucrose
15 mM	NaCl
50 mM	MES-NaOH
5 mM	$MgCl_2$

Buffers for Photoactivation of PSII membranes particles

Buffer Photo A (pH 6.0)	
0.3 M	Sucrose
35 mM	NaCl
50 mM	MES-NaOH