Thylakoid purification

Put a hand of leaves and about 100ml B1 in the blender, homogenize for 3x6s at the highest speed. Filter through 4 layers of cheese cloth. Centrifuge everything that passed the cloth for 10'at 1400g, 4°C (is 3600rpm in JA10 or GS3 big rotor).

Discard supernatant. Resuspend the pellet with a brush in buffer 2. Centrifuge 4000g for 10' at 4°C. Discard supernatant. Resuspend the pellet with a brush in buffer 3. Centrifuge 6000g for 10' at 4°C. Resuspend the pellet (thylakoids) in buffer 4.

Everything should be done as much as possible in the cold and dark. Samples should be quickly frozen in N_2 (l) and stored at -80°C.

Method mostly based on Berthold, 1981, FEBS. For BBY also see Caffarri, 2009, EMBO.

B1	Concentration stock	1L	941 ml water
0.4M sorbitol		72,868 g	
5mM $MgCl_2$	1M	5 ml	
20mM tricine/KOH pH 7.8	0.5M	40 ml	
5mM EDTA	0.5M	10 ml	
0.2mM Benzamidina	0.1M	2 ml	
1mM acido e amino caproic acid	0.5M	2 ml	

Last two are protease inhibitors and not strictly required.

Addition of EDTA is based on Casazza, 2001 (*Photosynthesis Research* **68**: 175-180, 2001).

B2	Concentration stock		0.5L	473 ml water
20mM tricine pH 7.8	0.5M	20 ml		
0.15M Sorbitol		13,66775 g		
5mM $MgCl_2$	1M	2.5 ml		
0.2mM Benzamidina	0.1M	1 ml		
1mM acido e amino caproic acid	0.5M	1 ml		
2.5mM EDTA	0.5M	2.5 ml		

B3	Concentration stock		0.6L	580.5 ml water
15mM NaCl	2M	4.5 ml		
5mM $MgCl_2$	1M	3 ml		
20mM Hepes pH 7.5	1M	12 ml		

B4	Concentration stock		100ml	97,75 ml water
0.4M sorbitol	30	7,2868 g		
15mM NaCl	2M	0.75 ml		
5mM $MgCl_2$	1M	0.5 ml		
10mM Hepes pH 7.5	1M	1 ml		

	1400g	3500g	4000g	6000g	40000g
JA10	3570		6037	7400	
JA14	3800		6444	7900	
JA17	3737	6000	6317	7739	17000