05-06-2025

We beginnen met het isoleren van pSI. Dit doen we in 2 stappen:

① Thykaloid isolation

② Sucrose gradient

Voor Stap 1 hebben we de volgende buffers nodig:

B1 (1 Liter)

0.4M sorbitol	72.87 g	+ 941 mL water
5mM $MgCl_2$ (0.5M)	5mL	
20 mM tricine/KOH pH 7.8 (0.5M)	40 mL	
5mM EDTA (0.5M)	10 mL	
0.2mM Benzamidine (0.1M)	2mL	
1mM aquado e amino caproic acid (0.5M)	2mL	

B2 (0.5 Liter)

20 mM tricine pH 7.8 (0.5M)	20 mL	+ 487 mL water
0.15 M sorbitol	13.66 g	
5mM $MgCl_2$ (1M)	2.5 mL	
0.2 mM Benzadimina (0.1M)	1 mL	
1mM acido e amino caproic acid (0.5M)	1 mL	
2.5mM EDTA (0.5M)	2.5 mL	

B3 (0.6 Liter)

15 mM NaCl (2M)	4.5 mL	+ 580,5 mL water
5mM $MgCl_2$ (1M)	3 mL	
20 mM Hepes pH 7.5 (1M)	12 mL	

B4 (100 mL)

0.4M sorbitol	7.2868 g	+ 97.75 mL water
15 mM NaCl (2M)	0.75 mL	
8 mM $MgCl_2$ (1M)	0.5 mL	
10mM Hepes pH 7.5 (1M)	1mL	

Voor Stap 2

Sucrose solution (50 mL)

0.5 M sucrose	8.557 g
20 mM HEPES pH 7.5	1 mL
0.06 % α-DM	0.03 g

Solution 1 (50 mL)

10 mM HEPES pH 7.5	0.5 mL
8 mM EDTA	0.073 g

Solution 2 (50 mL)

10 mM HEPES pH 7.5	0.5m

Solution 3 (50 mL)

10 mM HEPES pH 7.5	0.5m
1.2% α-DM	0.6 g