InsituPro VS protocol Arabidopsis whole mount 1

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System Configuration: 96 medium baskets

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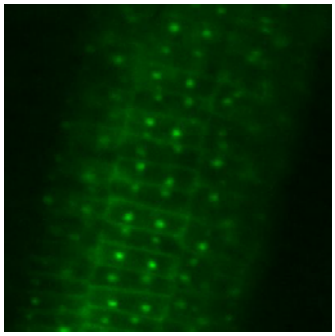


aPIN 1immunodetection after BFA treatment

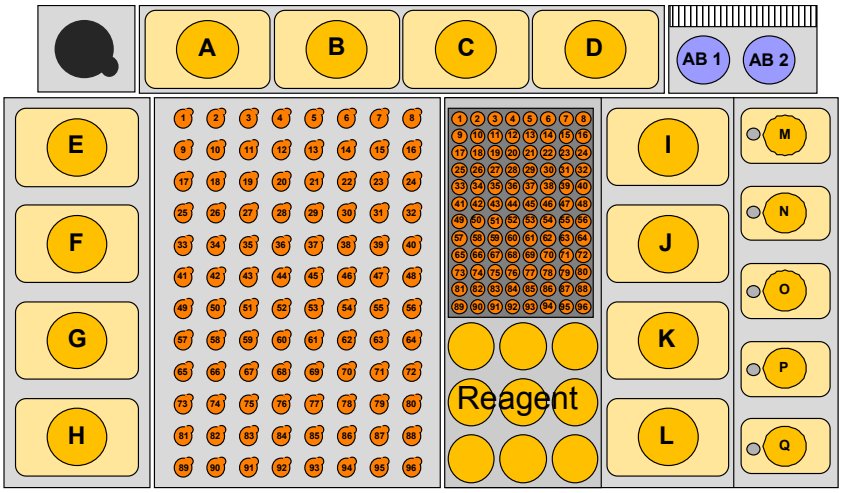
Description:

This protocol for immunodetection can be used for Arabidopsis seedlings (3-5 days old), previously fixed in 4% PFA in MTSB for 1 hour under vacuum. Fixed material is then loaded in the medium baskets, 10 to 15 seedlings per basket. In the ***InSituPro VS*** robot, the procedure started with several washes with MTSB, the cell wall is digested by treatment with Driselase and membranes are permeated with DMSO/Igepal. The samples are blocked with BSA and incubated with primary and secondary antibody. After several washes with MTSB, MTSB is exchanged by deionized water. Seedlings were transferred into slow antifade mounting media afterwards and fluorescence signals were inspected by confocal microscopy.
The amount of liquid that has to be delivered to basket is about 700 µl.

Step No.	Task	Time	Action	Proceeding
1	PrimeNeedle		12000	
2	SetTempReg		T0 (OFF)	
3	IncubateVT	15 min	700 MTSB/0.1%Triton->Specimen 5x	
4	IncubateVT	15 min	700 H2O/0.1% Triton->Specimen 5x	
5	SetTempReg		T1 (LOW)	37°C
6	IncubateVT	30 min	700 2% Driselase in MTSB/0.1% triton->Specimen	
7	SetTempReg		T0 (OFF)	
8	IncubateVT	15 min	700 MTSB/0.1%Triton->Specimen 5x	
9	IncubateVT	30 min	700 DMSO/igepal in MTSB/0.1% triton->Specimen 2x	
10	IncubateVT	15 min	700 MTSB/0.1%Triton->Specimen 5x	
11	IncubateVT	1 h	700 Blocking 2%BSA->Specimen	
12	SetTempReg		T1 (LOW)	37°C
13	IncubateVT	4 h	700 Probe->Specimen	
14	SetTempReg		T0 (OFF)	
15	IncubateVT	15 min	700 MTSB/0.1%Triton->Specimen 8x	
16	SetTempReg		T1 (LOW)	37°C
17	IncubateVT	5 min	700 GAR IgG Alexa 488 1/200->Specimen	
18	Wait	3 h		
19	SetTempReg		T0 (OFF)	
20	IncubateVT	15 min	700 MTSB/0.1%Triton->Specimen 5x	
21	IncubateVT	15 min	700 H2O->Specimen 5x	
22	PrimeNeedle	12000		

Specimen and Buffer loading Form

Method: **User:** **Date:**



Buffer Loading:

Vial	Buffer	Volume
A*	MTSB / 0.1% triton	
B*	MTSB / 0.1% triton	
C*	MTSB / 0.1% triton	
D*		
E*	H_2O / 0.1% triton	
F*	H_2O / 0.1% triton	
G*	H_2O / 0.1% triton	
H*		
I*	DMSO / igepal	
J*	2 % Blocking	

Vial	Buffer	Volume
K*	H_2O	
L*	2% Driselase	
M		
N		
O		
P		
Q / Q2		
AB		
AB 1	secondary antibody	
AB2		

Reagent

1		**4**		**7**	
2		**5**		**8**	
3		**6**		**9**	

Buffer printed in bold letters have to been put in during the Pause task !

Both vials on position AB (cooled rack) should be filled with antibody. The instrument needs up 80 ml antibody for the complete specimen rack!

Buffer amount can be reduced to 50 ml for positions E-H and I-L (labelled with *) by using the falcon adapters.

Buffers:

Buffer : MTSB / 0.1% T.	**pH: 7.0**
Substance	**Concentration**
PIPES	50 mM
EGTA	5 mM
$MgSO_4$	5 mM
adjust pH with KOH or H_2SO_4	
Triton X-100	0.1 %

Buffer : Driselase	**pH : 7.0**
Substance	**Concentration**
Driselase Powder	2%
in MTSB / 0.1% triton	

Buffer : DMSO / Igepal	**pH : 7.0**
Substance	**Concentration**
DMSO	10%
Igepal	3%
in MTSB / 0.1% triton	

Buffer : Blocking	**pH : 7.4**
Substance	**Concentration**
BSA	2%
in MTSB / 0.1% triton	

Buffer : primary AB	**pH : 7.4**
Substance	**Concentration**
Affinity purified AB	individual
in blocking solution	

Buffer : sec. AB	**pH : 7.4**
Substance	**Dilution**
Anti rabbit or mouse Alexa 488 (green) or 568 (red) conjugate	1/200
in blocking solution	

Other Tips and Tricks

Improved buffer:
We use acetylated BSA (Aurion) for blocking and antibody solutions. It is improving the signal/noise ratio.
Driselase should be prepared freshly. Do not try to dissolve it. Let the powder decant.
Temperature used for antibody incubation is 37°C