Preparation of nuclear fraction

Preparation of cytosolic and nuclear protein fractions. Protocol courtesy of Dr. Laszlo Bako, Umeå Plant Science Centre.

Procedure

1. Prepare protoplasts from 50 ml Arabidopsis thaliana cell culture according to the protocol of PEG transfection.
2. Resuspend protoplasts in 10 ml GH buffer and keep the solution on ice for 10 min.
3. To release nuclei add Triton X100 to a final concentration of 0.1%. Pipetting gently up and down several times with a plastic pipette might be necessary to lyse cells.
4. After 5min sediment nuclei by centrifugation at 1000 xg for 15 min at 4°C. Save supernatant as the cytoplasmic fraction. Wash the pelleted nuclei two times with GHT (GH+0.1% TX100) then finally resuspended in a suitable volume of extraction buffer + protease inhibitors.

Required solutions

GH BUFFER

Glycine	100mM
Hexylene glycol	0.1%
Saccharose	0.37M (4.7% w/v)
Spermine	0.3mM
Spermidine	1.0mM

pH 8.3 with $Ca(OH)_2$

Question? Please contact us at:

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