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Working

ROS staining for Arabidopsis (green fluorescent stain)

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ABSTRACT

This protocol is adapted from two publications describing fluorescent staining for ROS - Shin et al., 2005 and Orman-Ligeza et al. 2016 (citing Shin)

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Murashige & Skoog medium including B5 vitamins	M0231	Duchefa Biochemie
Costar® 6 Well Clear TC-Treated Multiple Well Plates, Individually Wrapped, Sterile	3516	Westnet
CM-H2DCFDA	C6827	Thermo Fisher Scientific

BEFORE STARTING

prepare sterile MS medium:
- 1/2 MS
- 0.5% sucrose
- 0.1% MES buffer
- pH 5.8 with KOH

Autoclave

prepare CM-H2DCFDA according to the supplier instructions

1 Sterilize the seedlings, germinate and transfer to for e.g. salt stress medium as described in the "Quantification of salt-induced changes in Root System Architecture in Arabidopsis" protocol - dx.doi.org/10.17504/protocols.io.zkqf4vw

2 Transfer the seedlings from agar plates into nutrient solution that is the same as agar plate composition (but without Dashin agar). You can use 24/12/6 well-plates - this will depend on your final seedlings size

3 After transferring the seedlings to liquid medium add DCF-DA so that the final concentration of the DCF-DA in the incubation medium is 50 uM

4 Wrap the well plate in aluminium foil (DCF-DA is light sensitive) and let the samples incubate for 30 min with gentle shaking.

5 Wash the seedlings with medium without DCF-DA

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6 Image the stain using fluorescence microscopy:
- 460–500 nm bandpass excitation
- 510– 560 nm bandpass emission

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