

# <sup>14</sup>C-paraquat Efflux Assay in *Arabidopsis* Mesophyll Protoplasts

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## Abstract

Weeds compete with crops for growth resources, causing tremendous yield losses. Paraquat is one of the three most common non-selective herbicides. To study the mechanisms of paraquat resistance, we need to trace the movement of paraquat in plants and within the cell. <sup>14</sup>C is a radioactive carbon isotope widely used to trace substances of interest in various biological studies, especially in transport analyses. Here, we describe a detailed protocol using <sup>14</sup>C-paraquat to demonstrate paraquat efflux in *Arabidopsis* protoplasts.

**Keywords:** <sup>14</sup>C-paraquat, Isotope, Protoplasts, Efflux transport, *Arabidopsis*

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## Background

Paraquat (PQ), a non-selective broad-spectrum herbicide, has been widely used in weed control. It mainly acts on plant green tissue and is relatively safe for soil microorganisms and plant roots (Huang et al., 2019). It competes for electrons from the photosynthesis system I (PS I) and reacts with oxygen, producing a large quantity of reactive oxygen species (ROS) that destroy the photosynthesis system and ultimately kill plants (Apel and Hirt, 2004). However, with the heavy use of PQ, resistant weeds have emerged (Hawkes, 2014). Through extensive studies of PQ-resistant weeds and *Arabidopsis*, several molecular mechanisms of PQ resistance have been unraveled. These include disrupted PQ transport that blocks it from reaching its target, PQ sequestration into the vacuole and apoplast, enhanced ability to scavenge ROS, and PQ detoxification (Chen et al., 2009; Fujita et al., 2012; Xi et al., 2012; Li et al., 2013; Luo et al., 2016; Lv et al., 2021; Xia et al., 2021; Huang et al., 2022; Nazish et al., 2022).

To study the function of the MATE transporter DTX6, we designed an experiment using  $^{14}\text{C}$ -paraquat and *Arabidopsis* protoplasts to confirm its PQ-exporting activity from cytosol to apoplast, which assisted us in revealing the PQ resistance mechanism via sequestration.

## Materials and Reagents

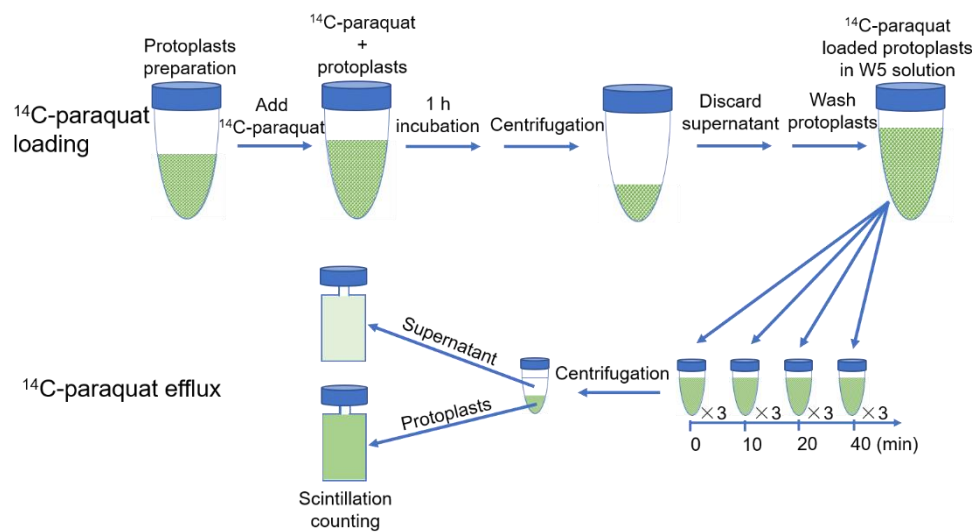
1. 50 mL centrifuge tube with round bottom
2. 2 mL centrifuge tube with round bottom
3. 70  $\mu\text{m}$  Nylon mesh (EMD Millipore, CLS431751)
4. 3–4 weeks old *Arabidopsis* plants
5. Scintillation fluid (Perkin-Elmer, Waltham, catalog number: 6013321)
6.  $^{14}\text{C}$ -paraquat (American Radiolabeled Chemicals Inc. ARC 1311). Store at  $-20\text{ }^{\circ}\text{C}$
7. NaCl (Sinopharm Chemical Reagent Co., Ltd., CHINA, CAS: 7647-14-5)
8.  $\text{CaCl}_2$  (Sinopharm Chemical Reagent Co., Ltd., CHINA, CAS: 10043-52-4)
9. KCl (Sinopharm Chemical Reagent Co., Ltd., CHINA, CAS: 7447-40-7)
10. MES (SIGMA, USA CAS: 1266615-59-1)
11. W5 solution (see Recipes)

## Equipment

1. Centrifuge with temperature control (Allegra X-22R Benchtop Centrifuge, Beckman Coulter, Indianapolis, USA)
2. Automated cell counter (IC1000, Shanghai RuiYu Biotech Co. Ltd., Shanghai, CHINA)
3. Ventilation cabinet (YKD-DAT004F, Wuxi Safoo Safety Equipment Co. Ltd., Wuxi, CHINA)
4. Scintillation vial (Perkin-Elmer, Waltham, USA)
5. Liquid scintillation counter (Tri-Carb 2910 TR, Perkin-Elmer, Waltham, USA)
6.  $-20\text{ }^{\circ}\text{C}$  freezer

## Procedure

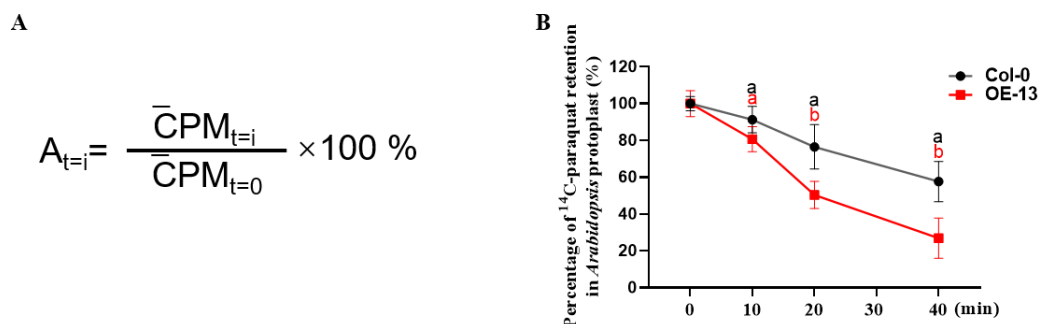
1. Isolate protoplasts according to Yoo et al. (2007) and suspend in cold W5 solution (see Recipes).
2. Count the protoplasts with a cell counter to estimate their concentration. Centrifuge at  $40 \times g$  and  $4\text{ }^{\circ}\text{C}$  for 2 min. Decant the supernatant and resuspend the protoplasts in cold W5 solution to approximately  $3 \times 10^6$  protoplasts/mL. This protoplast preparation is ready for  $^{14}\text{C}$ -paraquat loading (Figure 1).



**Figure 1. Experimental scheme of  $^{14}\text{C}$ -paraquat loading into and efflux out of *Arabidopsis* mesophyll protoplasts.**

- Thaw out  $^{14}\text{C}$ -paraquat (3.1 mM, 0.1  $\mu\text{Ci}/\mu\text{L}$ ) and dilute 10 times with W5 solution at room temperature.
- Add the same volume of diluted  $^{14}\text{C}$ -paraquat solution to the protoplast preparation, to obtain a final concentration of 3  $\mu\text{M}$   $^{14}\text{C}$ -paraquat. Incubate for 1 h at room temperature to load paraquat into the protoplasts.
- Centrifuge the protoplasts at  $40 \times g$  and  $4^\circ\text{C}$  for 2 min. Discard the supernatant with the unloaded  $^{14}\text{C}$ -paraquat and wash the protoplasts with 15 mL of cold W5 solution 2–3 times.
- Resuspend the protoplasts in 13 mL of W5 solution at room temperature and immediately initiate the paraquat efflux assay.
- At different time points (0, 10, 20, and 40 min) after W5 solution resuspension, transfer 1 mL of protoplast suspension into a 2 mL round-bottom centrifuge tube. Take at least three replicates at each time point.
- Immediately centrifuge the aliquoted samples ( $40 \times g$ , 2 min,  $4^\circ\text{C}$ ). Transfer the supernatant to a scintillation vial. Resuspend the protoplast pellet in 1 mL of W5 solution and transfer it to a scintillation vial. Then add 5 mL of scintillation fluid to each sample. Detect radioactivity on a liquid scintillation counter.
- Analyze and present data as shown in Figure 2.

Note:  $^{14}\text{C}$ -paraquat waste was disposed according to the University's safety management of isotopes.



**Figure 2. Data analyses and presentation.**

(A) Calculation of  $^{14}\text{C}$ -paraquat retention in protoplasts at different time points.

$t=i$ : time of  $^{14}\text{C}$ -paraquat protoplasts efflux (0, 10, 20, and 40 min).

$\text{CPM}_{t=i}$ : mean value of  $^{14}\text{C}$ -paraquat radioactivity retention in protoplasts at different time points.

$\text{CPM}_{t=0}$ : mean value of  $^{14}\text{C}$ -paraquat radioactivity retention in protoplasts at the initial time.

$A_{t=i}$ : percentage of  $^{14}\text{C}$ -paraquat radioactivity retention in protoplasts at different time points relative to the initial time.

**(B)** Paraquat retention in the protoplasts, measured in the Col-0 and *DTX6* overexpression line (OE-13) (Xia et al., 2021). Paraquat retention was defined as a percentage of the  $^{14}\text{C}$ -paraquat loaded in the protoplasts. Values are mean  $\pm$  SD ( $n = 3$ ). Different letters indicate significant differences ( $P < 0.05$ ; one-way ANOVA).

## Recipes

### 1. W5 solution

154 mM NaCl  
125 mM  $\text{CaCl}_2$   
5 mM KCl  
2 mM MES  
pH 5.7

## Acknowledgments

This protocol was adapted from previous work (Zhang et al., 2014; Qin et al., 2021); we would like to thank the authors of these studies for their protocols.

## Competing interests

No conflict of interest declared.

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