

## Determination of Luciferase Activity in *Arabidopsis* Seedling

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**[Abstract]** There are different direct and indirect methods available to study gene expression in plant systems. In this protocol we describe a modified expression assay using transgenic plants expressing the luciferase gene under the control of phosphate transporter PHT1;1 promoter. This assay was originally optimized for analyzing the repression of PHT1;1 promoter in response to arsenate As (V) which can be adapted to study the kinetics of transcriptional regulation of any gene in response to biotic or environmental stimuli measuring Luciferase activity in *Arabidopsis thaliana*.

### Materials and Reagents

1. Transgenic *Arabidopsis* seedlings expressing Luciferase fused to promoter of interest (*i.e.* PHT1;1:Luc in pGWB435 binary vector)  
*Note: Vector information can be found at: <http://shimane-u.org/nakagawa/pgwb-tables/2.htm>.*
2. KH<sub>2</sub>PO<sub>4</sub> (Sigma-Aldrich, catalog number: P5655)
3. Sodium arsenate dibasic heptahydrate (Sigma-Aldrich, catalog number: A6756)
4. 30 mM arsenate (see Recipes)
5. 0.36 mM D-Luciferin (Sigma-Aldrich, catalog number: L9504) (see Recipes)
6. Johnson media (see Recipes)

### Equipment

1. Growth chamber [16-h-light/8-h-dark regime (24 °C/21 °C)]
2. White 96-well plates (Greiner Bio-One GmbH, catalog number: 655074)
3. MicroAmp optical adhesive Film (Life Technologies, Applied Biosystem®, catalog number: 4311971)
4. Luminometer (centro LB 960) (Berthold Technologies)

## Software

1. Mikro Win 2000 software

## Procedure

1. Sow the transgenic seeds minimum (24 plants for each treatment) in Petri dish containing Johnson media (0.6% agar) with 1 mM phosphate and incubate in growth chamber with 16-h-light/8-h-dark regime (24 °C/21 °C) for 7 days.
2. Transfer the seedlings to Johnson media (0.6% agar) lacking phosphate and leave for 3 days.
3. Add 200 µl of Johnson liquid media (without agar) to White 96-well plate and then transfer single seedlings to each well by placing upside down.

*Note: Since PHT1;1 is prominently expressing in root we submerged the seedlings upside down.*

4. Add D-Luciferin substrate (50 µM final concentration) to each well and incubate for 1 h.
5. Measure the initial luminescence *i.e.* Luminescence (0).
6. Add arsenate to each well (30 µM final concentration) and cover the plate with MicroAmp optical adhesive Film (refer to Reference 1 to understand the behavior of PHT1;1 in response to Arsenic).
7. Measure the luminescence of the plate in every 10 min interval with the luminometer using Mikron Win 2000 software (counting time for each well is two seconds).
8. Calculate the percentage of luciferase activity (%LUC activity) as follows:  

$$\%LUC \text{ activity} = [(Luminescence(n) - Luminescence(0)) / Luminescence(0)] * 100$$
 Luminescence (0) = Luminescence at time zero  
 Luminescence (n) = Luminescence at 2<sup>nd</sup> and consecutive readings  
 Once we calculate the %LUC activity for each time point, we have to calculate the average Luminescence of all the independent plants at each time point.
9. Luciferase activity can be also determined in *Nicotiana benthamiana* (*N. benthamiana*) using 1 cm discs of agro infiltrated leaves transferred to a microplate and proceed as described above.

## Recipes

1. 30 mM arsenate  
 Dissolve 468 mg of sodium arsenate dibasic heptahydrate in 50 ml water  
 Filter sterilize

2. 0.36 mM D-Luciferin
  - Mix 1 mg of D-Luciferin with 8 ml dH<sub>2</sub>O
  - Warm at 80-90 °C for 30 to 40 sec to dissolve it
  - Add dH<sub>2</sub>O to 10 ml
  - Filter sterilize (0.25 µm)
  - Stored at -20 °C
3. Johnson media

Macronutrientes	Stock	ml stock/L
KNO <sub>3</sub> (4 °C)	100 g/L	6.06 ml
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O (4 °C)	100 g/L	9.446 ml
MgSO <sub>4</sub> ·7H <sub>2</sub> O (4 °C)	100 g/L	2.46 ml

Micronutrientes	g for 100 ml stock 1000x	ml stock/L
KCl (-20 °C)	0.378 g	1 ml
H <sub>3</sub> BO <sub>3</sub> (-20 °C)	0.15457 g	
MnSO <sub>4</sub> ·H <sub>2</sub> O (-20 °C)	0.0845 g	
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (-20 °C)	0.0575 g	
CuSO <sub>4</sub> ·5H <sub>2</sub> O (-20 °C)	0.01248 g	
H <sub>2</sub> MoO <sub>4</sub> (-20 °C)	0.00161 g	

	g for 200 ml stock 500x	ml stock/L
FeSO <sub>4</sub> ·7H <sub>2</sub> O (4 °C) (ajustar pH a 3.5 con H <sub>2</sub> SO <sub>4</sub> diluido) Proteger de la luz	0.1112 g	2 ml

	Stock	stock/L
Mio-inositol (4 °C)	50 g/L	2 ml

MES	0,5 g/l
1% sacarosa	10 g/L
0.5% sacarosa	5 g/L

Make volume to 1 L using Milli Q water  
Adjust the pH to 5.7 with KOH

0.6% Agar

After Autoclave add phosphate  $\text{KH}_2\text{PO}_4$

1 M  $\text{KH}_2\text{PO}_4$ : 6.8 g/50 ml (filter sterilize)

Add 1 ml/L to get final concentration of 1 mM

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