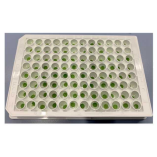

Mar 22, 2021

Protocol for chemiluminescence based detection of ROS production in tomato

Parvinderdeep S Kahlon[1], Remco Stam[1]

[1]Chair of Phytopathology, TUM School of Life Sciences Weihenstephan, Freising-Weihenstephan, Germany

1 *Works for me* dx.doi.org/10.17504/protocols.io.beeejbbe

Stam Lab
Tech. support email: **remco.stam@tum.de**

Parvinderdeep S Kahlon
Chair of Phytopathology, TUM School of Life Sciences Weihens...

SUBMIT TO PLOS ONE

ABSTRACT

This assay is based on luminol and horseradish peroxidase-dependent chemiluminescence detection of reactive oxygen species (ROS) production in plant leaf discs with some modification to make it suitable for tomato, specifically for wild tomato *Solanum chilense*. We tried non-buffered (Water) and buffered systems (MES and MOPS) to evaluate the robustness to detect ROS production in *S. chilense* leaf discs. Buffering the leaf discs with 20mM MOPS at pH 7.5 during the assay resulted in the best outputs with high reproducibility.

ATTACHMENTS

ROSbuffered.png

DOI

dx.doi.org/10.17504/protocols.io.beeejbbe

PROTOCOL CITATION

Parvinderdeep S Kahlon, Remco Stam 2021. Protocol for chemiluminescence based detection of ROS production in tomato. **protocols.io** https://dx.doi.org/10.17504/protocols.io.beeejbbe

KEYWORDS

luminol, Plate Reader, Tomato, ROS production, Solanum chilense

CREATED

Mar 31, 2020

LAST MODIFIED

Mar 22, 2021

PROTOCOL INTEGER ID

34982

GUIDELINES

Avoid any mechanical stress while pipetting the solutions in and out of the wells.

MATERIALS TEXT

Materials required: Biopsy punch/leaf punch (4mm diameter), 96 wells plate (opaque white-walled with solid bottom).

Instrument required: 96-well plate luminometer (Luminoskan Ascent/Tecan plate reader).

Chemical required: 50mM L-012, 50mM horseradish peroxidase (HRP) dissolved in 50% glycerol, 20mM MOPS pH 7.5 (adjust with 1N NaOH).

HRP mix: 10µM HRP, 10µM L012 in 20mM MOPS pH 7.5.

1 Make leaf discs (8 leaf discs per genotype) with biopsy punch and incubate in 200µl of 20mM MOPS in 96- well plate overnight.

2 Remove the buffer the next day and add 75µl of HRP mix to each well.

3 Read the plate for detecting luminescence in 1-5 minutes intervals at the luminometer for the initial 10 minutes to adjust the baseline.

4 Add 25µl in 4X concentration of the desired elicitor (flg22 in this case) in 4 wells and add water/solution used for dissolving elicitor as a negative control in another 4 wells for individual genotype.

5 Read the plate for detecting luminescence in 1-5 minutes intervals for 60-180 minutes.

6 Normalize the data first to an average of initial 5-10 minutes read (before elicitation) and then to the negative control.

Citation: Parvinderdeep S Kahlon, Remco Stam (03/22/2021). Protocol for chemiluminescence based detection of ROS production in tomato. https://dx.doi.org/10.17504/protocols.io.beeejbbe