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We use this protocol and it's working

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Protocol for chemiluminescence based detection of ROS production in tomato V.1

Parvinderdeep S
Kahlon¹,

Remco
Stam¹

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

Stam Lab

Tech. support email: remco.stam@tum.de

Parvinderdeep S Kahlon

Chair of Phytopathology, TUM School of Life Sciences Weihens...

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](#)

GUIDELINES

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

MATERIALS

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.

Keywords: luminol, Plate Reader, Tomato, ROS production, *Solanum chilense*

- 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.