Plant:

• Arabidopsis suspension cultured cell (L*er*)
(May, M.J. and Leaver, C.J. (1993) PlantPhysiol. 103, 621-627)

Bacterial pathogen:
• *Pseudomonas syringae* pv. *tomato* DC3000 *avrRpm1*

Materials:
• Nunc 96-deep well plate #260252 for incubation
• 8-channel pipet (for small volume)
• 8-channel pipet (for middle volume)
• 8-channel electronic pipet (for large volume)
• 96-well plate for absorbance measuring

• Evans blue solution (1% Evans blue in water)
• Elution solution (50%Methanol, 1%SDS)
• VH medium:
  MS
  3 % Sucrose
  0.5 mg/L NAA (naphthaleneacetic acid)
  0.05 mg/L 6-BAP (6-benzyl amino purine)

Bacteria Mixture:
  VH medium(hormone free) 27.5ml
  0.5M MES(pH5.0) 2.86ml (final conc. 14.3 mM)
  Pst(OD2) 10ml (final OD0.2)

Protocol:
Aliquot 58.5 µl of suspension cells by 8-channel pipet using top cut tip
↓
Add 0.5 µl of chemicals in each well by 8-channel pipet
(We used DMSO as solvent of chemicals and it has no effect on HR cell death up to at least 0.5 %.)
↓
Mix well by hand tapping
↓
Incubate for 1 hour with occasional shaking
↓
Add 41 µl volume of Bacteria Mixture (Final volume is 100 µl)
↓
Incubate on shaker for about 20 hrs
↓
Add 5ul of Evans Blue solution (Final 1%)
↓
Incubate for 1hour with occasional shaking

↓
Add 1 ml of H_2O for washing by 8-channel electronic pipet
↓
Remove 1 ml H_2O with electronic pipet after waiting for several minutes
↓
Repeat washing 3 times
↓
Add 400 µl of Elution solution by electronic 8-channel pipet
↓
Float plate in 55 ^oC water bath and incubate for 20 min
↓
Aliquot 150 µl elution solution in 96-well plate
↓
Add 50 µl of samples to 150 µl elution solution in 96-well plate (4 times dilution)
↓
Measure OD_{595} absorbance using plate reader

Note: Japan Patent 2008-088491