THE SAINSBURY LABORATORY
STANDARD OPERATING PROCEDURE

TheSainsburyLaboratory TSL

TITLE: *In planta* co-immunoprecipitation (Summer School protocol)

APPLIES TO STAFF IN: Sainsbury Laboratory and Students at the summer school

HEALTH & SAFETY INFORMATION INCLUDED: YES

REFERENCE No: TSL037	**VERSION No: 1.0**
DATE EFFECTIVE: August 2017	**REVIEW DATE: August 2019**
AUTHOR: Joe Win	**APPROVED BY: Simon Foster**

1 PURPOSE OF PROCEDURE/METHOD AND ITS SCOPE

To purify plant proteins that associate with the effectors expressed *in planta*. *Nicotiana benthamiana* will be used as a model host plant.

2 EQUIPMENT NEEDED

Pipettes
Pipette tips
Centrifuge
Anti-FLAG M2 affinity gel
GTEN: 10% glycerol, 25 mM Tris pH 7.5, 1 mM EDTA, 500 mM NaCl
Extraction Buffer: GTEN, 2% w/v PVPP, 10 mM DTT, 1x protease inhibitor cocktail (Sigma), 0.5% Igepal
Immunoprecipitation (IP) buffer: GTEN, 0.5% Igepal
Conservation Buffer: 5 mM Tris pH 7.5
Frozen leaf powder obtained from *N. benthamiana* leaves expressing the effectors
Dry ice

3 STEPS IN PROCEDURE

- **Wear lab coat, safety glasses, and gloves. Observe local safety rules.**
- **Be careful not to make long contact with dry ice when handling the tube with frozen leaf powder**
- **Dispose of all biologically contaminated waste according to local safety rules, e. g., use the 'kill box' for re-useable items and blue box for disposable items. Sharp items must be enclosed in yellow-cap plastic bottles.**

3.1 Weigh out 1 g leaf powder and resuspend it in 2 ml ice-cold extraction buffer. Vortex thoroughly until the solution is homogenous, and keep on ice.

3.2 Centrifuge at full speed in a microcentrifuge for 10 min at 4°C. Transfer the supernatant into syringe and filter through 0.45 µm membranes. Keep the filtrate (~1 ml) in a new tube and place on ice.

3.3 Re-suspend the anti-FLAG gel by tapping the side of the vial several times and mix with a cut pipette tip.

3.4 Pipette enough gel (20 µL per sample) into a 1.5 ml Eppendorf tube. Centrifuge at 800 × *g* for 1 min and carefully remove the supernatant (**take care not to aspirate the gel**).

3.5 Re-suspend the gel in 1 ml of IP buffer.

3.6 Centrifuge at 800 × *g* for 1 min and remove the supernatant.

3.7 Re-suspend the gel to 2x original volume with IP buffer and add 40 µL of diluted gel to the leaf extract prepared above (step 2).

3.8 Incubate the gel and the leaf extract at 4°C for 1-3 h on a rotating mixer.

3.9 Centrifuge at 800 × *g* for 30 s. Discard supernatant and add 1 ml of fresh IP buffer. Repeat four more times but always leave about 50 µl at the bottom of the tube to avoid aspirating the beads. After the last wash, remove as much supernatant as possible without touching the beads. Beads are now ready for trypsin digestion and mass spectrometry.

4 RISK STATEMENT

This activity is low risk and does not involve handling any hazardous chemicals except EDTA at very low levels (1 mM) and small quantities of DTT and IGEPAL CA-630.

All individuals using this procedure will be shown the risk assessment and given appropriate information, instruction and training in the risks and precautions necessary, including the use of any personal protective equipment required.

SOP HEALTH RISK ASSESSMENT

[1] Activity:	***In planta* co-immunoprecipitation**
[2] Location of activity:	The Sainsbury Laboratory and Chris Lamb Training Suite
[3] Who is involved:	Science staff in The Sainsbury Laboratory and participants to the TSL Summer School
[4] Frequency of activity:	Variable
[5] Duration of Activity:	7-10 days

[6] Chemical Hazard Name:	**Hazard Statements**	**Route of Exposure***	**Quantity Used**
Ethylenediaminetetraacetic acid (EDTA) 	H319- Causes serious eye irritation	S, I	Not more than 2 ml at 1 mM
Dithiothreitol (DTT) 	H302- Harmful if swallowed H315- Causes skin irritation H319- Causes serious eye irritation H412- Harmful to aquatic life with long lasting effects	S, I	<1g
IGEPAL® CA-630 	H302- Harmful if swallowed H315- Causes skin irritation H318- Causes serious eye damage H410- Very toxic to aquatic life with long lasting effects	S, I	<1 ml

[7] Details of biological agents of risk to human health:	**GMRA Number**
None	

[8] Other Hazards: Please ■ as necessary

Hot or Cold Burns	☒	Ionising Radiation**	☐	Ultra Violet or Infra Red	☐
Dust	☐	Noise	☐	Pollen Sensitizer	☐

Repetitive Action	☐	Extreme Cold Environment (< 0°C)	☐	Lifting / Manual Handling	☐
Asphyxiation	☐	Cuts	☐	Electrical	☐
Slips / trips / falls	☐	Display Screen Equipment	☐	Other (give details)	☐

[9] Control Measures: Please ■ as necessary

Fume Cupboard	☐	Microbiological Safety Cabinet	☐	Total Containment Cabinet	☐
Ventilated Bench	☐	Spill Tray	☐	Trained personnel only	☒
Signs	☐	Reduce frequency/alternate activity	☐	Reduce duration of activity	☐
Sub divide a load	☐	2 man lift of equipment	☐	Not for more than 1 hour	☐
Regular, short breaks	☐	Alternate activities	☐	Other (give details)	☐

[10] Personal Protection: Please ■ as necessary

Lab coat	☒	Safety Glasses	☒	Face Shield	☐
Goggles	☐	Gloves	☒	Thermal Protective Gloves	☐
Ear defenders	☐	Other (give details)	☐		

[11] Is personal monitoring and/or health surveillance required? Yes ☐ No ☒

Details:

[12] Restrictions: Please ■ as necessary

No lone working	☐	Not to be left unattended	☐	Named persons only	☐
In restricted area	☐	Not by new or expectant mothers	☐	Under constant supervision	☐
Not by under 18's	☐	Other (give details)	☐		

[13] Level of Residual Risk: Please ■ as necessary

Low	☒	Medium	☐	High	☐

Name of Assessor:	Date:
Simon Foster	21st July 2017

* Route of exposure; S = skin, I = ingestion, B = inhalation

Activities involving new or expectant mothers and young persons also require additional risk assessment.

1 DOCUMENTATION

Links to relevant H&S information on intranet or internet

Reference any relevant manuals

Link to: JIC Chemical Tables:
http://intranet/infoserv/support/QualityAssurance/Chemical_Tables_SOPs.htm

Link to: Good Laboratory Practice in the Use of Chemicals:
http://intranet/infoserv/support/Safety/Chemical/GLP_Chems.htm

Link to Biological and GM Safety:
http://intranet/infoserv/Support/Safety/Biological/index.htm

Link to Laboratory Waste Disposal:
http://intranet/infoserv/Support/Safety/Waste/index.htm

Win J et al., (2011) Purification of Effector–Target Protein Complexes via Transient Expression in Nicotiana benthamiana. In Plant Immunity, McDowell JM (ed) pp 181-194-194. Humana Press

Petre B et al., (2015) Candidate Effector Proteins of the Rust Pathogen Melampsora larici-populina Target Diverse Plant Cell Compartments. Mol Plant Microbe Interact 28: 689-700

6 RELATED PROCEDURES

Other relevant SOPs

7 NOTES

8 APPENDICES

Chemical	**Ethylenediaminetetraacetic acid** (EDTA)	Sigma: 431788 CAS: 60-00-4
Hazard Statement	H319 Causes serious eye irritation.	
Precautionary Statement	P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
Handling	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.	
Storage	Store in cool place. Keep container tightly closed in a dry and well-ventilated place.	
Disposal	Dispose of in a chemically compatible container; ensure liquids are placed in a container designed for liquids. Label and place in the collection tray for “Waste Not Suitable for Bulking-Up” in the Chemical Waste Store. Where possible, use the container in which the chemical was supplied. Follow the user instructions for Using the Chemical Waste Store. Consult the Chemical Safety Officer or your Lab Manager for more information.	
Spillage	Wear appropriate personal safety equipment; lab coat, gloves and safety glasses and absorb spill with inert material e.g. vermiculite and place in suitable container and dispose of in Chemical Waste Store.	

Chemical	**Dithiothreitol** (DTT)	Sigma Aldrich D0632 CAS:3483-12-3
Hazard Statement(s)	H302 Harmful if swallowed. H315 Causes skin irritation. H319 Causes serious eye irritation.	
Precautionary Statement(s)	P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
Handling	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.	
Storage	Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature: 2 - 8 °C	
Disposal	Dispose of in a chemically compatible container; ensure liquids are placed in a container designed for liquids. Label and place in the collection tray for “Waste Not Suitable for Bulking-Up” in the Chemical Waste Store. Where possible, use the container in which the chemical was supplied. Follow the user instructions for Using the Chemical Waste Store. Consult the Chemical Safety Officer or your Lab Manager for more information.	
Spillage	Wear appropriate personal safety equipment; lab coat, gloves and safety glasses and clean up spills with blue roll or spill tamer kit. Put in suitable container and dispose of in Chemical Waste Store.	

Chemical	**Igepal® CA-630**	Sigma I8896 CAS: 9002-93-1
Hazard Statement(s)	H302 Harmful if swallowed. H315 Causes skin irritation. H318 Causes serious eye damage. H410 Very toxic to aquatic life with long lasting effects. .	
Precautionary Statement(s)	P280 Wear eye protection/ face protection. P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.	
Handling	Avoid contact with skin and eyes. Avoid inhalation of vapour or mist	
Storage	Store in cool place. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.	
Disposal	Dispose of in a chemically compatible container; ensure liquids are placed in a container designed for liquids. Label and place in the collection tray for “Waste Not Suitable for Bulking-Up” in the Chemical Waste Store. Where possible, use the container in which the chemical was supplied. Follow the user instructions for Using the Chemical Waste Store. Consult the Chemical Safety Officer or your Lab Manager for more information.	
Spillage	Wear appropriate personal safety equipment; lab coat, gloves and safety glasses and clean up spills with blue roll or spill tamer kit. Put in suitable container and dispose of in Chemical Waste Store.	