RISK ASSESSMENT – TASK BASED
IGEM 2016

Location:	**Building Number:**	**Date:**	**Assessed By:**	**Health & Safety Representative:**
Room W301, Medical Building	181	February, 2016	Amber Willems Jones	Vincé Kalangi

Description of Activity: **Inducing protein expression in E. coli** SWP No. C2V2 Inducing Protein Expression	
Is there past experience with the Activity that may assist in the risk assessment? Incidents & Near-hits, Incident Investigations, Workplace Inspections, Training, Standards, Legislation & Codes, Uni Guidance Material, Existing Controls, Industry Standards.	NO

1. TASK	**2. RISK**	**3. Estimated RAW RISK SCORE** C x E x L	**4. CONTROLS**	**5. RESIDUAL RISK SCORE** C	E	L	CxExL	**6. RESIDUAL RISK**
Decontamination of waste	Hypochlorite (Diversol) causes skin burns and damage to eyes.	3 x 2 x 1	Prepare disinfectant solutions in fumehood.	3	2	0.1	0.6	LOW
	Ethanol is flammable	1 x 2 x 1	Avoid naked flames	1	2	0.1	0.2	LOW
Working with antibiotics	May cause an allergic skin reaction	15 x 6 x 1	Personal protective equipment, training	15	6	1	4.5	LOW
Working with IPTG	Harmful by inhalation, in contact with skin and if swallowed	3 x 2 x 1	Personal protective equipment, work in well ventilated area	3	2	0.1	0.6	LOW
Bench top centrifuge	Samples unbalanced, manual handling of rotors	15 x 3 x 1	Adequate training and induction to equipment	15	3	0.1	4.5	LOW
	TOTAL	149		TOTAL			10.4	LOW RISK

Name and Signature of Laboratory Head/Supervisor or Delegate	Amber Willems Jones	Date	
Name and Signature of Persons Performing Activity or Task		Date	

Number and Title	C2V2 Inducing Protein Expression
Name of Laboratory/ Department	The University of Melbourne IGEM Team Laboratory/ Department of Biochemistry
Author, Date Prepared and Date of Review	**Author:** Ella Bocquet-Gaylard **Date:** 22/2/2016 **Updated :** February 2016, **Review by**: February 2018
Introduction	Inducing protein expression in E.coli
Principals/ Scope	This SWP describes the steps to follow in order to induce protein expression in E.coli, where there is an IPTG-inducible promotor.
Risk Management	Risk assessments have been prepared and are available on the Task Based Risk assessment attached to the SWP. **Raw Risk:** low Residual **Risk:** low
Safety Management	**Hazards:** Wear PPE **Risk Controls:** Low Risk
License/ Permits	N/A
Training/ Competency	All team members must be inducted to the use of any Equipment used.
Equipment	Bench top Centrifuge Shaking Incubator
Protocol **Step 1** **Step 2** **Step 3** **Step 4** **Step 5**	Pick a single colony from a freshly streaked plate of the expression host containing the recombinant vector. Inoculate the picked colony in 4mL of LB containing the appropriate antiboiotic (use 1μL of antibiotic for 1 mL of LB). Grow the culture for 4-8 hours at 37°C with shaking (200 rpm). After incubating, transfer 1mL of the culture to 25mL of LB with the appropriate antibiotic added. Grow the culture overnight at 37°C with shaking. Don't leave the culture for any longer than 16 hours. Ensure that the cold room incubator is available for overnight expression. Note that you need to use the cold room incubator to get the temperature down to 16°. Spin the overnight cultures to remove β-lactamase and resuspend the pellet in 800mL fresh medium (with antibiotic added) in a 2L flask. Do not vortex cells.

Step 6	Grow the cells at 37°C with shaking and monitor the OD. When the OD reaches 0.6, cool the culture to 16°C in ice water. Note: For more detailed instructions for this procedure see 'A4 Measuring OD Protocol'.
Step 7	Add 800μL of 1M IPTG solution (filter sterilized) to the culture and induce overnight at 16°, 200 RPM. Add the Aerotop seals if using Ultra Yield Flasks.
Step 8	Harvest the cell pellet using a centrifuge for 20 minutes at high speed and store the cell pellet at -20°C. Do not vortex cells.
Controls/ Calibration	N/A
Waste Disposal	Laboratory benches and surfaces used must be decontaminated with 80% ethanol and all bacterial waste must be treated with Hypochlorite before being disposed of in the biohazard bin.
Emergency Procedures	**First aid measures** Eye contact: Immediately flush eyes with plenty of water for at least 20 minutes and get medical attention. Skin contact: In case of contact, immediately flush skin with plenty of water for at least 20 minutes. Inhalation: Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Get medical attention. Ingestion: Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call medical doctor or poison control centre immediately. If there are any issues with the centrifuge spin, immediately cancel the spin.
References	2014 University of Melbourne IGEM Team Protocols
Authorised By	Amber Willems Jones