

Mammalian Cell Invasion Assay

No	Description/Details of Steps in Activity	Hazards	Possible Accident / Ill Health & Persons-at-Risk	Existing Risk Control (Mitigation)	Severity	Likelihood (Probability)	Risk Level	Additional Risk Control
1	1. Prepare overnight cultures of BL21, and BL21+pSB1C3-inv plasmid (Inoculate 1 colony in 3mL LB, grow O/N in shaking incubator)	Biological exposure; breakage of glassware	Handling of bacteria outside of a Class I Biohazard Safety Cabinet risks generating aerosol particles which may be inhaled or ingested	Internal training is compulsory. Conduct routine bacteria work at the lab bench over absorbant materials; wear suitable PPE (e.g. lab coat, covered shoes, gloves); decontaminate equipment and/or small spills with 70% ethanol or DeCon90.	1	1	2	
2	2. Seed 0.5mL of 5x10 ⁵ /cm ³ HepG2 cells per well in 24-well plates overnight, grow in CO ₂ incubator. Check to see if cells are 90% confluent.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
3	Grow bacteria under anaerobic conditions for 2, 4, and 6 hours, taking out aliquots at these timepoints and incubating on ice. Remove DMEM media, wash cells with 500ul sterile 1XPBS. Do not pipette up and down when washing. Add 400ul fresh warmed DMEM media+FBS without Pen-Strep. Work well by well to prevent drying out. Take bacteria out of the shaking incubator, and mix by pipetting up and down. (Measure OD ₆₀₀ of 10X dilution. Dilute bacteria with PBS to 10 ⁹ cells/mL. Spin down 1mL and resuspend in PBS. Prepare 2X dilution and 4X dilution.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens, Biological exposure	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury.	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
4	Inoculate 100ul of 2X dilution and 4X dilution of bacterial suspension to give an Moi of 200 or 100. Make sure pipette enters media, and move while releasing suspension. When done, swirl plate to ensure even distribution of bacteria. Infection for 3 hours in CO ₂ incubator.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens, Biological exposure	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury.	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
5	Remove supernatant, and wash once with PBS. Add 400uL fresh, warmed media with Kanamycin at 1000ug/mL. After a further 1 hour of infection, remove media, wash cells 2X with 500ul PBS and lyse cells with 500ul PBS/0.2% Triton X-100. Perform serial dilution of the cell cultures to 10 ³ and plate out duplicates to determine number of intracellular bacteria. Keep plates at room temperature, transfer to incubator to grow overnight. Count number of colonies and calculate CFU in the morning.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens, injury due to improper usage of centrifuge and fingers. Exposure to antibiotics	Exposure to antibiotics which are toxic	Internal training is compulsory for centrifuge use and the centrifuge key. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand. When using the centrifuge, ensure centrifuge is balanced and rotor is placed correctly, and that all tubes are capped tightly. Close centrifuge properly and ensure that there are no funny sounds when centrifuge is running. Decontaminate waste using Presept tablets (dilute to 10% solution of actived bleach to kill cells) and let the bleach decontaminate for half an hour before discarding with plenty of water and dilution.	1	1	2	