ANNEXURE 1

OPTIMISED METHOD FOR MYCOLIC ACID-LIPOSOME IMMOBILISATION

1.1 Liposome preparation

1. Aliquot MA in 100µg quantities
2. Make fresh PC and cholesterol stock solutions of 10mg/ml in cold chloroform
3. Add necessary quantities of PC and cholesterol stock solutions to one vial of MA (100µg)
4. Dry the mixture in a heat block at 85°C under a steady stream of nitrogen gas.
5. Add 2ml PBS containing 0.025% (w/v) sodium azide and 1mM EDTA (PBS/AE) to the dried lipids
6. Melt the lipids at 85°C in a heat block for ten minutes or until no residual flakes are visible
7. Vortex the solution at 100% for one minute
8. Sonify with a micro probe sonicator set at pulsed, 20 duty cycles, output level two for one minute
9. Vortex the solution at 100% for one minute

1.2 Liposome immobilisation

1. Achieve a stable baseline with PBS/AE for at least five minutes
2. Aspirate cells, add 50µl of 20µg/ml CPC in PBS/AE and incubate for ten minutes
3. Wash the cells five times each with 60µl PBS/AE and add 25µl of PBS/AE
4. Incubate for at least five minutes and until a stable baseline has been achieved.
5. Add 25µl of the liposome solution and incubate for ten minutes
6. Wash cells five times with 60µl PBS/AE, and immediately wash with five times 60µl 1mg/ml saponin in PBS/AE. Note: Saponin concentration should be titrated first to optimal blocking capacity concentration
7. Incubate for ten minutes and until a stable baseline has been achieved
8. Wash five times with 60µl PBS/AE and add a final 25µl PBS/AE

1.3 Interaction analysis and regeneration

1. Enter a baseline event as soon as a stable baseline has been achieved after the PBS/AE wash. Baseline should be monitored for at least five minutes
2. Add 25µl of the desired test sample at an appropriate dilution to the cells and enter an association event
3. Incubate for five to ten minutes depending on association velocity
4. Wash five times with 60µl PBS/AE to initiate dissociation and enter a dissociation event
5. Continue dissociation for five minutes before regenerating the cells as follow:
6. Wash three times with 50µl 95% ethanol
7. Follow immediately with three times 80µl PBS/AE and five times 60µl PBS/AE over one minute
8. Wash five times with 60µl 12.5M KOH and incubate for two minutes
9. Follow with three times 80µl PBS/AE and five times 60µl PBS/AE over one minute

ANNEXURE 2

OPTIMISED METHOD FOR GANGLIOSIDE-LIPOSOME IMMOBILISATION

2.1 Liposome preparation

1. Aliquot G_{M1} in 100µg quantities
2. Make fresh PC stock solution of 1mg/ml in cold chloroform
3. Add necessary quantity of PC stock solution to one vial of G_{M1} (100µg)
4. Dry the mixture in a heat block at 85°C under a steady stream of nitrogen gas.
5. Add Tris based buffer (50mM Tris, 200mM NaCl, 3mM NaN_3, 1mM Na_2EDTA) to the dried lipids to a concentration of 0.5mg/ml
6. Vortex the solution at 100% for one minute
7. Sonify with a micro probe sonicator set at pulsed, 20 duty cycles, output level two for one minute
8. Vortex the solution at 100% for one minute

2.2 Liposome immobilisation

Ganglioside-liposomes are immobilised with residual HCl remaining in the cells after regeneration. Therefore the regeneration protocol should be followed first and then immobilisation can be continued.

1. Aspirate the HCl solution remaining after regeneration and add 50µl of the liposome solution
2. Incubate for ten minutes before washing cells four times with 60µl Tris buffer, four times with 60µl 10mM NaOH and another five times with 60µl Tris buffer finishing with a 25µl Tris buffer addition
3. Incubate for five minutes and until a stable baseline has been achieved

2.3 Interaction analysis and regeneration

1. Enter a baseline event as soon as a stable baseline has been achieved after the Tris buffer wash. Baseline should be monitored for at least five minutes
2. Add 25µl of the desired test sample at an appropriate dilution to the cells and enter an association event.
3. Incubate for five to ten minutes depending on association velocity

4. Wash five times with 60µl Tris buffer to initiate dissociation and enter a dissociation event
5. Continue dissociation for five minutes before regenerating the cells as follow:
6. Wash four times with 60µl absolute ethanol
7. Follow immediately with seven times 100µl water, five times 60µl KOH and incubate for two minutes
8. Wash seven times with 100µl water and follow with three times 2M HCl and four times 20mM HCl
9. Achieve a stable baseline for at least five minutes before aspirating for the next round of immobilisation