



# Institute of Food Research

## Protocol from the *Salmonella* group



## Extracting bacterial RNA from infected tissue culture cells

### Cell culture and infection

1. Mammalian cell growth conditions (media, temperature, %CO<sub>2</sub>), MOI, mode of infection (invasive or complement-opsonised bugs) and lysing procedure will have to be optimized for each cell line. Bacterial uptake, growth and viability will have to be optimized for each application.
2. Cultivate J774-A.1 cells (ATCC TIB 67) in RPMI-1640 medium (Gibco) supplemented with 10% Fetal Bovine Serum (v/v final), 4 mM L-glutamine (final concentration) and 20 mM HEPES (final concentration); this is referred to as complete medium.
3. All incubations are performed at 37°C, 5% CO<sub>2</sub>.
4. For each assay, cells should be grown until 80% confluence is reached in 75cm<sup>2</sup> flasks.
5. For each extraction of *Salmonella* RNA, a total of 12-tissue culture flasks per time point were used.
6. NB: batches of all components should be checked for LPS contamination.
7. Cells are infected with *S. Typhimurium* SL1344 at a multiplicity of infection 10:1 or as desired.
8. Bacteria are diluted 1/10 from an overnight culture in LB+0.3M salt and grown to an OD of 1.2 (optimum conditions for invasion). Bacteria are then diluted in HEPES-buffered RPMI (without serum) to the correct concentration for invasion.
9. Cells should be washed (10 ml/flask) twice in HEPES-buffered RPMI (without serum) before infection. Bacteria are subsequently seeded on the J774-A.1 cells (10ml/flask). To increase the uptake of *Salmonella*, plates are centrifuged at 1000g for 5min at room temperature, and this is defined as time 0h.
10. After 0.5h incubation, extracellular bacteria are killed by changing the medium to killing medium (killing medium containing 30 µg/ml gentamicin) and incubating for 1h. For continued incubations (i.e. for a further 2h, 6h or 10h), killing medium is replaced by complete medium containing only 5 µg/ml gentamicin (10 ml/well) half an hour after addition.

For extracting RNA from bacteria grown in mammalian cells see protocol:

8) RNA extraction from bacteria inside tissue culture cells

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