



Institute of Food Research

Protocol from the *Salmonella* group



RNA extraction from bacteria inside tissue culture cells.

1. All solutions are bought from Sigma's assortment of RNase free buffers and chemicals, and from Promega.
2. At each time-point, infected mammalian cells are lysed for 30 min, on ice, in 0.1% SDS, 1% phenol pH 4.3 (Sigma; order code P-4682), 19% ethanol in water, enough to cover the cell layer. (This lysis step may need to be optimised for different infection protocols).
3. Lysates are collected in cold Eppendorf or 50 ml falcon tubes, depending on the volume, and pellets were collected by centrifugation (10 min, 14000 rpm, 4°C for microcentrifuge tubes or 20 min at 4000-8000 rpm at 4°C for falcon tubes).
4. The supernatant is removed, pellets re-suspended and pooled. This is repeated until all the bacteria from a particular time point are present in one microcentrifuge tube. The pellet can then be stored at -80°C if desired.
5. Pellets are used for RNA preparation and labelling. As only a small amount of bacterial RNA is obtained by this protocol it is usually necessary to use the 'Labelling protocol for reduced amounts of RNA'.