# Choline Uptake Assay in Bacterial Cells

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**[Abstract]** Choline is a methylated nitrogen compound that is widespread in nature. It is a precursor of several metabolites that perform numerous biological functions and it is predominantly used for the synthesis of essential lipid components of the cell membranes. Since there is no evidence that prokariotes can synthesize choline *de novo* and because choline uptake from exogenous sources is energetically more favorable than *de novo* synthesis, bacteria have evolved different uptake mechanisms for choline transport across the bacterial membrane. This protocol describes an easy and high sensitive method to assess choline uptake in bacteria using as tracer [$^3$H]-choline chloride. The protocol was originally intended for *Brucella abortus* but could be applied for any bacteria with the corresponding modifications depending on the bacteria growth requirements (composition of the culture medium, temperature for growth, *etc.*). It can be useful to determine the choline uptake ability of several bacterial species under different growth conditions.

## Materials and Reagents

1. *Brucella abortus* or the bacteria species you want to test
2. Minimal medium (MM) such as M9 or equivalent
   In the case of *Brucella abortus* we use Gerhardt-Wilson (GW) minimum medium (Gerhardt, 1958)
3. Choline Chloride ([Methyl-$^3$H]-, 250 µCi (9.25 MBq)) (PerkingElmer, catalog number: NET109250UC)
4. [$^3$H]-choline/cold choline chloride (1:100)
5. Liquid Scintillation (liquid Optiphase HISAFE 3) (PerkingElmer, catalog number: 1200-437)

## Equipment

1. Microplate reader or Spectrophotometer
2. Liquid scintillation spectrometer (Gemini BV, model: WinSpectral™ 1414)

 

## Procedure

1. For radioactive choline uptake analyses, cultures of bacteria grown in an appropriate minimal medium (MM) at mid log phase were harvested, washed three times with ice-chilled MM and adjusted to an optical density at 600 nm ($OD_{600}$) of 0.5 with fresh MM.
   *Note: Bacterial culture is harvested at $OD_{600}$= 1-1.2 that correspond to a mid log phase for Brucella under this growth conditions.*
2. Reactions were initiated by addition of [$^3$H]-choline chloride (80.6 Ci/mmol) to a final concentration of 3.3 µM, incubated at 37 °C and aliquots (around 300 µl) were taken at different time points (0 to 30 min).
3. Samples were immediately chilled on ice, washed five times with the same volume of ice-chilled MM, centrifuged at 10,000 *x g*, 15 min at 4 °C and cell pellets were resuspended in 500 µl of scintillation liquid.
4. The radioactivity in the cell pellet was determined with a liquid scintillation spectrometer.
5. To assess uptake kinetics at different choline concentrations, bacteria were incubated 7 min at 37 °C in MM with a mix of [$^3$H]-choline/cold choline (1:100) ranging from 6.25 x $10^{-2}$ µM to 64 µM (total choline concentration) and incorporated radioactivity in the cell pellet was determined as describe above.

## Acknowledgments

This protocol was adapted from Herrmann *et al.* (2013).

## References

1. Gerhardt, P. (1958). The nutrition of brucellae. *Bacteriol Rev* 22(2):81-98.
2. Herrmann, C. K., Bukata, L., Melli, L., Marchesini, M. I., Caramelo, J. J. and Comerci, D. J. (2013). Identification and characterization of a high-affinity choline uptake system of *Brucella abortus*. *J Bacteriol* 195(3): 493-501.

 