

Protocol for Quorum Sensing Induction for toxins production in *Clostridium difficile* NTCC 13307



Protocol Code: TI_ind

Materials:

- Brain Heart infusion medium supplemented with thioglycolate and vitamin K
- Columbia with hydrolyzed blood agar supplemented with thioglycolate and vitamin K
- Falcon of 15 mL
- Anaerobic chamber at 37°C
- Spectrophotometer
- Promega kit, SV Total RNA Isolation System
- Promega kit, M-MLV Reverse Transcriptase
- Sybr green, Roche

Procedure

Day 1. Pre culture

1. Prepare the tester cells, a single colony was selected from a Columbia agar with blood and vitamin K agar plate after 48h of incubation and cultured in 5 ml of fresh BHI medium with thioglycolate and vitamin K overnight (16 to 18 h).

Day 2.

1. The overnight culture (optical density at 600 nm [OD600] 1.4 to 1.5) was diluted 1:100 with fresh BHI medium with thioglycolate with vitamin K and incubated for 3 h (early log phase).
2. For the quorum-sensing assay, take 50 µl of washed low-density early-log-phase tester cells and add to a fresh medium containing 1 ml of AIP extract and 2.95 ml of fresh BHI medium with thioglycolate and vitamin K.
3. Incubate for 48 h anaerobically at 37°C.
4. As a control, the tester cells were added to 3.95 ml of fresh reduced BHI medium and incubated under the same conditions as the treated cells.

Solution	BHI (mL)	Early-log-phase (µL)	AIP (µL)

5. Take samples at 0h, 16h, 24h and 48h.
6. From the samples do a RNA extraction following described by Promega "SV Total RNA Isolation System".
7. After the RNA extraction do RT-PCR to convert ARN to ADNc following the protocol describe by Promega "M-MLV Reverse Transcriptase". This DNAc could be stored at - 20°C.
8. At the end of the incubation period, the supernatant was tested for toxin expression by real-time PCR following the protocol "Sybr green Roche".

The primers for the qPCR are:

- 5'TGCCAGAAGCTCGCTCCACA3' (forward)
- 5'TGCACTTGCTTGATCAAAGCTCCA3' (reverse) for *tcdA*
- 5'GTGTAGCAATGAAAGTCCAAGTTTACGC3' (forward)
- 5'CACTTAGCTCTTTGATTGCTGCACCT3' (reverse) for *tcdB*

References:

Darkoh, C., DuPont, H. L., Norris, S. J., & Kaplan, H. B. (2015). Toxin synthesis by *Clostridium difficile* is regulated through quorum signaling. *MBio*, 6(2), e02569-14.

Darkoh, C., Kaplan, H. B., & DuPont, H. L. (2011). Harnessing the glucosyltransferase activities of *Clostridium difficile* for functional studies of toxins A and B. *Journal of clinical microbiology*, 49(8), 2933-2941.