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## Immunostaining of *Bodo saltans* V.2

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**We use this protocol and it's working**

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## Abstract

This protocol is used in our Laboratory in Liverpool to perform IF on Bodo saltans cells.

## Culture conditions

- 1 *Bodo saltans* was cultured in a cerophyl-based medium enriched with 3.5 mM sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_4$ )<sup>1</sup>. Cultures were incubated at 22 °C in T25 tissue culture flasks containing 20 ml of media bacterized with *Klebsiella pneumoniae subsp. Pneumoniae* (ATCC® 700831).

## Immunostaining

- 2 Please, follow the steps below to prepare cells for immunostaining.
  - 2.1 Filter the culture through 100 and 8 µm filter.
  - 2.2 Harvest the cells by centrifugation at 1200 × g for 12 mins at 19 °C.
  - 2.3 Wash the cells with PBS and centrifuge as described above.
  - 2.4 Dissolve the pellet in 15 µl of PBS and mix with the same volume of low melting temperature agarose (eg. Thermo Fisher Scientific) in a single well of a 96-well plate. Let it set for a few seconds.
  - 2.5 Add 200 µl of 4% PFA and incubate at room temperature for 10 minutes or at 4 °C overnight.
  - 2.6 Wash with PTX (PBS + 0,1% TritonX) 4 times, 30 mins each wash.
  - 2.7 Block in 5% FBS+PTX overnight at 4 °C.
  - 2.8 Incubate with primary antibody (1:100, diluted in PTX + 5% FBS), for 5 hours at room temperature or overnight at 4 °C.
  - 2.9 Rinse and wash 3 times for 1 hour with PTX at room temperature.

- 2.10 Incubate with secondary antibody (1:1000, diluted in PTX + 5% FBS) overnight at 4°C.
- 2.11 Rinse and wash 3 times for 1 hour with PTX at room temperature. During 2<sup>nd</sup> wash add Hoechst 33342 (Thermo Fisher, 1:2000) for 10 minutes. This will be rinsed away with the 3<sup>rd</sup> wash.
- 2.12 Remove the agarose from the well with clean forceps and place it on a microscope slide.
- 2.13 Add a drop of a mounting medium (eg. Vectashield, Vector Laboratories), and flatten the agarose as much as you can using the coverslip.
- 2.14 Proceed with either fluorescence or confocal imaging.

2.15

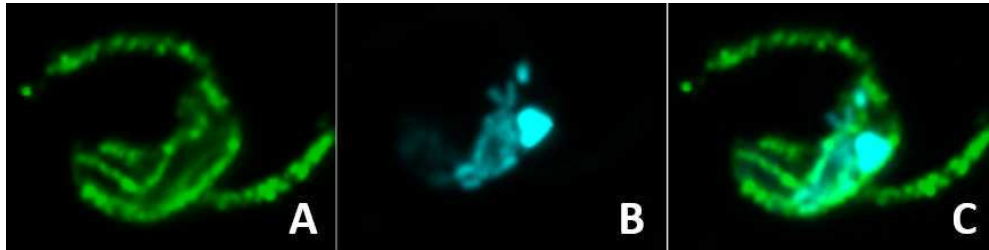


Figure 1: Confocal image of a single fixed *Bodo saltans* stained with beta-tubulin antibody (clone KMX-1, gift from Dr. Jack Sunter (Oxford Brooks) and Prof. Keith Gull (Oxford University)). A) Beta-tubulin. B) DNA of the same of *Bodo* cell (nucleus, kinetoplast and intracellular bacteria) stained with Hoechst 33342 . C) Overlay of the two channels.

2.16

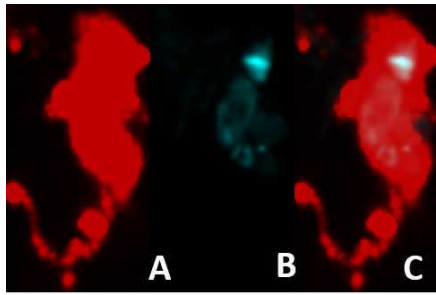


Figure 2: Confocal image of a single fixed *Bodo* cell stained with beta-actin antibody (clone D6A8, Cell Signalling Technology). A) Beta-actin. B) DNA the same of *Bodo* cell (nucleus, kinetoplast and intracellular bacteria) stained with Hoechst 33342 C) Overlay of two channels.

## References

- 3 Gomaa Fatma, Li ZuHong, Docampo Roberto, Girguis Peter, E. V. *Bodo* saltans culture protocol V.2. Protocols.io (2018). doi:10.17504/protocols.io.sh6eb9e