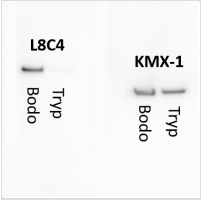

May 03, 2022

Protein extraction, quantification, and western blot for *Bodo saltans*

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dx.doi.org/10.17504/protocols.io.5qpvobqodl4o/v1

Symbiosis Model Systems | Bodo protocols

Ewa Chrostek

This protocol is used in our Laboratory in Liverpool to work with proteins from Bodo saltans and other kinetoplastids (eg. trypanosomes).

DOI

dx.doi.org/10.17504/protocols.io.5qpvobqodl4o/v1

Ewa Chrostek, Mastaneh Ahrar, Gregory Dd Hurst 2022. Protein extraction, quantification, and western blot for Bodo saltans. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvobqodl4o/v1

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Culture conditions

1 *Bodo saltans* was cultured in a cerophyl-based medium enriched with 3.5 mM sodium phosphate dibasic (Na_2HPO_4)[1]. Cultures were incubated at 22 °C in T25 tissue culture flasks containing 20 ml of media bacterized with *Klebsiella pneumoniae subsp. Pneumoniae* (ATCC® 700831).

Protein extraction and quantification

1.1 Centrifuge 20 ml of 1 week old culture at 1200 × g for 12 mins at 19 °C to pellet

the cells.

1.2 Dissolve pellet in lysis buffer[2]: 150 mM NaCl, 1% Triton X-100, 50 mM TrisHCl (pH8), 5 mM EDTA. Supplement lysis buffer with cOmplete Protease Inhibitor Coctail (Roche) (1 tablet per 10 ml of solution) and Benzonase nuclease (Sigma) immediately before extraction to prevent protein degradation and to decrease viscosity of extract, respectively.

1.3 Incubate extract on ice for 30 mins.

1.4 Centrifuge at 8000 × g to pellet debris.

1.5 Collect supernatant.

1.6 Quantify protein (eg. using the Pierce™ BCA Protein Assay Kit, (Thermo Fisher) according to the kit instructions).

Western blot

2 For western blot, adjust protein concentrations so that they are equal between samples.

2.1 1. Separate protein extracts on polyacrylamide gels (eg. Bolt Bis-Tris Plus, Thermo Fisher).

2.2 2. Transfer proteins to PVDF membrane (eg. using mini blot module (Thermo Fisher)).

2.3 3. Block the membrane for 30 mins at room temperature in 5% skimmed milk diluted in wash buffer (PBS with 0.1% Tween20).

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2.4 4. Incubate the membrane overnight at 4 °C with primary antibodies (1:100 times diluted in 5% milk in wash buffer).

2.5 5. Wash the membrane 3 × 10 mins with wash buffer (PBS with 0.1% Tween 20).

2.6 6. Incubate the membrane for 1 hour at room temperature with Horseradish Peroxidase (HRP)-labelled secondary antibodies (1:5000 dilution in 5% milk in wash buffer).

2.7 7. Wash the membrane 3 × 10 mins with wash buffer (PBS with 0.1% Tween 20).

2.8 8. Develop the signal with HRP substrate (eg. Immobilon western kit, Merk) and scan for chemiluminescent signal (eg. with ImageQuant LAS4000 or Bio Rad imaging system).

2.9

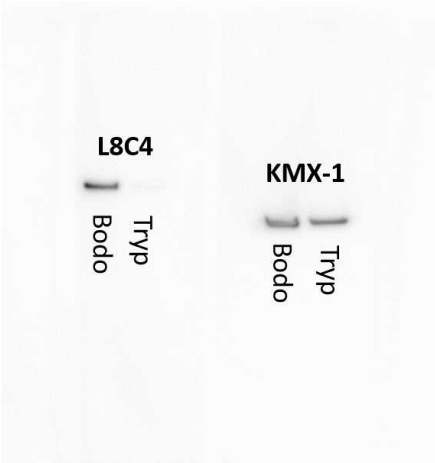

Figure 1. Immunoblot showing *Bodo saltans* and a trypanosome *Herpetomonas muscarum* (Tryp) protein extracts reacting with L8C4 and KMX-1 antibodies (kind gift from Dr. Jack Sunter (Oxford Brooks) and Prof. Keith Gull (Oxford University)).

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Citation: Ewa Chrostek, Mastaneh Ahrar, Gregory Dd Hurst Protein extraction, quantification, and western blot for Bodo saltans https://dx.doi.org/10.17504/protocols.io.5qpvobqodl4o/v1