Trypanosoma brucei Antigen Expression and Purification

Cascade protein purification protocol

Protocol for the expression of recombinant proteins under the lac-promotor, and purification of strep-tagged II proteins.

Protein expression
1. Inoculate a pre-culture of the Escherichia coli Rosetta-strain in a 50 ml Greiner tube from glycerol stock (pre-culture volume >1/100th of final culture volume), in LB medium with appropriate antibiotics (100 mg/L Ampicillin, 25 mg/L Chloramphenicol).
2. Grow overnight at 37˚C in a shaking incubator.
3. Inoculate the final culture (500 ml -1000 ml) 1/100th with the pre-culture.
4. Grow at 37˚C in a shaking incubator to an OD600 of about 0.4-0.5.
5. When density is reached, cold shock the culture on ice for 30 min.
6. Induce the culture with 0.5 mM IPTG.
7. Incubate culture at 23˚C in a shaking incubator (at least 180 rpm) for 16-18h.
8. Spin down the culture in large centrifuge tubes (5000 rpm, 10 min at 4˚C) or Greiner tubes (4700 rpm, 10 min at 4˚C). From this point on always keep the samples and buffers on ice or at 4˚C.
9. Resuspend the cells in ice cold Buffer A+(lysis), use ~1/20th of culture volume.
10. Lyse cells with the Bandelin Sonopuls ultrasonic homogenizer (Am 30%, pulse 1.0 s 2.0 s) for 2x 5 minutes while keeping the sample on ice.
11. Centrifuge the lysate in the pre-cooled ultracentrifuge (16000 rpm, 30min at 4˚C).
12. Filter the lysate through a 0.45 μm syringe filter into a clean, cold 50 ml Greiner tube.

Protein purification
1. Prepare the Strep-tactin beads. Load the desired column volume of 50% Strep-tactin bead solution to a gravity column (Strep-Tactin® Sepharose® 50% suspension, Iba Cat.no: 2-1201-010). Bed volume= max 1/100th of lysate volume. Let the liquid run through the column by gravity alone, do not apply pressure. Collect 1 mL fractions of each step and store on ice.
2. Wash/equilibrate the column with 30-40 column volumes of ice cold Buffer A (storage).
3. Add the cell lysate to the column.
4. Wash the column with 30-40 column volumes (12 ml) of ice cold Buffer A+ (high salt wash).
5. Equilibrate column with 1 ml of ice cold Buffer A (storage).
6. Elute the protein 4x with 100-200 μl ice cold Buffer B+(elution) (50 μl for 125 μl beads, 100μl for 25 μl beads, 200μl for 500μl beads).
7. Run each Cascade prep on a 10% SDS PAGE gel.
8. Measure protein concentrations of the flow-through and washing fractions with the Nanodrop (A280). The real Cascade concentration is ~0.75x the measured concentration.
9. Measure the protein concentration of the eluted fractions using the Roti®-Nanoquant protein quantitation assay. Cascade can be stably stored at 4˚C for a few weeks, or with 50% glycerol at -20˚C for a few months. Best for long term storage: Snap freeze in liquid nitrogen, store at -80˚C.

Protein Quantitation assay

This protocol for the Roti®-Nanoquant protein quantitation assay is a modification by Carl Roth GmbH + Co. KG of Bradford’s protein assay [4], [5], and is used to measure protein concentrations in protein samples.

1. Dilute the 5 times concentrated Roti®-Nanoquant (Carl Roth, K880) with dH2O to create the working solution.
2. Prepare the BSA calibration standards. First pipette the standard of 100 and 10 μg BSA/mL (bold lines), prepare all other standards using these two stock solutions.

<table>
<thead>
<tr>
<th>BSA (μg/mL)</th>
<th>μL (from dil.)</th>
<th>μL dH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>20 μL (from 10 μg/mL)</td>
<td>180</td>
</tr>
<tr>
<td>2.5</td>
<td>50 μL (from 10 μg/mL)</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>100 μL (from 10 μg/mL)</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>40 μL (from 100 μg/mL)</td>
<td>360</td>
</tr>
<tr>
<td>25</td>
<td>50 μL (from 100 μg/mL)</td>
<td>150</td>
</tr>
<tr>
<td>50</td>
<td>100 μL (from 100 μg/mL)</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>150 μL (from 100 μg/mL)</td>
<td>50</td>
</tr>
<tr>
<td>100</td>
<td>200 μL (from 400 μg/mL)</td>
<td>600</td>
</tr>
</tbody>
</table>
3. Pipet the following volumes into clean cuvettes:
   - 200 µL of each standard + 800 µL Roti®-Nanoquant working solution.
   - 200 µL of each protein sample + 800 µL Roti®-Nanoquant working solution.

4. Mix by inverting repeatedly.

5. Pipette dH2O in the reference cuvette.

6. Determine the OD590 and OD450 of all the standards and samples with dH2O as reference.

7. Plot OD590/OD450 against the BSA protein concentration from the standards (in µg/mL).

8. Use the calibration curve to calculate the protein amount in the sample. Lower detection limit: 0.2 µg protein (c=1 µg/mL).

9. Induction of protein expression
   1. Inoculate 200µL overnight E. coli culture into a 14-mL tube containing 5 mL of liquid LB and the appropriate antibiotics.
   2. Grow cells for a few hours at 37ºC, shaking at 250-300rpm. Make sure the tubes are tilted.
   3. Watch the turbidity. Once the culture reaches an OD600 of 0.4-0.6, take out 2 mL of the culture. Measure the actual OD600.
   4. Aliquot the equivalent of 1 mL of cells at OD600=0.8 in a 1.5-mL microfuge tube, i.e., volume in mL=0.8/OD600 of sample.
   5. Spin down at maximum speed for at least 1 min. Carefully remove all of the supernatant. This is the uninduced sample. Store the cells at -20ºC.
   6. Add 3µL of 1M IPTG to the remaining culture. Continue shaking at 300rpm overnight but at a lower temperature of 23ºC.
   7. The next day, measure the OD600.
   8. Spin down two tubes containing the equivalent of 1mL of cells at OD600=0.8 and remove the supernatant. These are your induced samples — one tube will be used to test for expression and the second for solubility. Store the cells at -20ºC.

10. Testing for expression
    9. For each construct, take the tube of uninduced and 1 tube of induced cells and resuspend each in 100µL of 1x SDS-PAGE sample buffer.
   10. Boil the samples for 10 min (98 ºC), then cool down to room temperature.
   11. Centrifuge for 5 min at maximum speed at room temperature.
   12. Analyse 10µL of each sample using SDS-PAGE.

13. Testing for solubility
    13. Take the remaining tube of induced cells and resuspend in 50µL of B-PER.
   14. Incubate at room temperature for 10 minutes.
   15. Spin down in a microcentrifuge at maximum speed for 10 min at 4ºC.
   16. Carefully transfer all of the supernatant into a new microfuge tube. Add 50µL of 2x SDS-PAGE buffer. This is the soluble fraction.
   17. Resuspend the pellet in 100µL of 1x SDS-PAGE buffer. This is the insoluble fraction.
   18. Boil the samples for 10 min, then cool down to room temperature.
   19. Centrifuge for 5 min at maximum speed at room temperature.
   20. Analyse 15µL of each sample using SDS-PAGE, with western blotting if necessary.