Concentration of Giardia supernatant proteins

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Abstract

This protocol provides an efficient and rapid way to concentrate proteins present in supernatants using Vivaspin columns (3,000 MWCO).

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Before start

Prepare a 25 mM Ambic (Ammonium bicarbonate) solution:

19.77 g Ambic in 10 ml Ultrapure H2O.

Protocol

BCA assay

Step 1.

Assess protein concentration before protocol.

PROTOCOL

. Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) with Sypro straining

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NOTES

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See BCA assay Protocol in Protocol 'Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) with Sypro straining'.

Protein sample preparation

Step 1.1.

Load proteins with loading buffer (containing loading dye) in a 3:1 ratio in Eppendorf tubes, then boil samples for 5 min at 100°C.

Protein migration by electrophoresis

Step 1.2.

Load 20 µl of protein samples and a protein standard molecular weight marker in gels placed in a vertical gel tank system filled with 1x running buffer. Run gels at 100 volts for 2 hours at room temperature.

AMOUNT

20 µl Additional info: Protein samples

Protein fixation on gel

Step 1.3.

After electrophoresis, immerse gels in a fixing solution bath for 30 min on a rotary shaker. (fixation 1/2)

Protein staining with SYPRO solution

Step 1.4.

Immerse gels in 40 ml of pre-diluted SYPRO solution and incubate overnight, protected from the light on a rotary shaker at room temperature.

AMOUNT

40 ml Additional info: Pre-diluted SYPRO

Protein wash with washing buffer

Step 1.5.

Wash gels with a washing solution for 30 min on a rotary shaker at room temperature, in a container protecting gels from the light.

SAFETY INFORMATION

Collect SYPRO solution in a specific container waste and follow MSDS and health and safety regulation to discard.

Protein wash with ultrapure water

Step 1.6.

Immerse gels in ultrapure water and wash for 5 min at room temperature on a rotary shaker, in a container protecting gels from the light. (wash 1/2)

SAFETY INFORMATION

Collect washing buffer in a properly labeled container and follow health and safety regulation to discard.

Reading gels

Step 1.7.

Read gels under UV machine.

SAFETY INFORMATION

Wear required protecting gear and discard gels in a acrylamide-specific container to be discarded following health and safety regulation.

Protein fixation on gel

Step 1.8.

Repeat the fixation one more time; immerse gels in a fixing solution bath for 30 min on a rotary shaker. (fixation 2/2)

Protein wash with ultrapure water

Step 1.9.

Immerse gels in ultrapure water and wash for 5 min at room temperature on a rotary shaker, in a container protecting gels from the light. (wash 2/2)

SAFETY INFORMATION

Collect washing buffer in a properly labeled container and follow health and safety regulation to discard.

Protein transfer onto vivaspin columns

Step 2.

Transfer sample in Vivaspin column and centrifuge 30 minutes at 12,000 rcf.

NOTES

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Make sure the tube is orientated as required and shown in the Vivaspin protocol (VivaProducts).

Protein wash #1

Step 3.

Discard liquid waste and add 1 ml of 25 mM Ambic and centrifuge 30 minutes at 12,000 rcf.

AMOUNT

1 ml Additional info: 25 mM Ambic

NOTES

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Make sure the tube is orientated as required and shown in the Vivaspin protocol (VivaProducts).

Protein wash #2

Step 4.

Discard liquid waste and add 1 ml of 25 mM Ambic and centrifuge 30 minutes at 12,000 rcf.

AMOUNT

1 ml Additional info: 25 mM Ambic

Protein wash #3

Step 5.

Discard liquid waste and add 1 ml of 25 mM Ambic and centrifuge 30 minutes at 12,000 rcf.

AMOUNT
1 ml Additional info: 25 mM Ambic

NOTES
Kevin Tyler 04 Jan 2018

This step is optional and is for samples that were in media containing phenole red

Protein concentration

Step 6.

Discard liquid waste and add 50 µl of 25 mM Ambic, incubate at room temperature for one hour and centrifuge at 3,000 rpm for 2 minutes.

AMOUNT
50 µl Additional info: 25 mM Ambic

NOTES
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Make sure to flip the column into clean cap before adding Ambic, see Vivaspin columns protocol.

Storage and sample verification

Step 7.

Run BCA assay and SDS-PAGE to check on protein concentration and protein degradation respectively. Store samples at -20C.

PROTOCOL

. Proteomics Analysis

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NOTES
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See protocols 'Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) with Sypro straining' and 'Proteomics Analysis'.

Sample preparation

Step 7.1.

Dilute the amount of protein needed, taking into account complexity, into 25mM AmBic. Small Eppendorf tube preferred.

Detergent treatment

Step 7.2.

Add 10µL of 1% (w/v) RapiGest (0.05% (w/v) final).

AMOUNT
10 µl Additional info: 1% (w/v) RapiGest

Detergent treatment

Step 7.3.

Heat at 80°C, 10minutes, vortex briefly at 5min.

TEMPERATURE
80 °C Additional info: Heat

Detergent treatment

Step 7.4.

Spin quickly to return liquid to the bottom of the tube.

Reduction

Step 7.5.

Add 10 µL of a 9.2mg/mL solution of DTT (3 mM final). Vortex mix.

AMOUNT
10 µl Additional info: 9.2mg/mL solution of DTT

Reduction

Step 7.6.

Incubate for 60°C, 10minutes.

TEMPERATURE
60 °C Additional info: Incubation

Reduction

Step 7.7.

Cool to RT and quickly spin to return liquid to the bottom of the tube.

Alkylation

Step 7.8.

Add 10 µL of a 33mg/mL solution of iodoacetamide (9 mM final). Vortex.

AMOUNT
10 µl Additional info: 33mg/mL solution of iodoacetamide

Alkylation

Step 7.9.

Incubate at RT, **IN THE DARK** for 30min.

Digestion

Step 7.10.

Add trypsin to 50:1 protein:trypsin ratio. Incubate 12-16h (overnight) at 37°C.

TEMPERATURE
37 °C Additional info: Incubation

Mass spec analysis

Step 7.11.

Analyze peptide mixtures on either nanoACQUITY-nLC system (Waters MS technologies) or Ultimate 3000 nano system (Thermo Fisher Scientific) followed by an LTQ-Orbitrap Velos (ThermoFisher Scientific) mass spectrometer or a Q-Exactive mass spectrometer (Thermo Fisher Scientific).

Warnings

Ambic is hazardous for the environment, check MSDS sheet to handle and discard waste.