Semi-quantitative analysis of western blot signals

Anna F. Zeitler, Luisa F. Jiménez Soto

Abstract

The protocol was creating adapting components from the manual from Bio-Rad for the analysis software ImageLab. Data analyzed using the protocol here described have appear in the following publications:

Zeitler AF, Gerrer KH, Haas R, Jiménez-Soto LF. Optimized semi-quantitative blot analysis in infection assays using the Stain-Free technology. J MicrobiolMethods. 2016 Jul;126:38-41. doi: 10.1016/j.mimet.2016.04.016. Epub 2016 May 3.PubMed PMID: 27150675.
Jiménez-Soto LF, Haas R. The CagA toxin of Helicobacter pylori: abundant production but relatively low amount translocated. Sci Rep. 2016 Mar 17;6:23227. doi: 10.1038/srep23227. PubMed PMID: 26983895; PubMed Central PMCID: PMC4794710.
and in the title 'Host cell resistance to CagA translocation is as variable as Helicobacter pylori'. Zeitler et al 2017 accepted in Matters journal.

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Protocol

Prepare a acrylamide gel containing Trichlorethanol (TCE)

Step 1.

Link to single-gel protocol published

REAGENTS
2,2,2- Trichlorethanol T54801-100G by Sigma

Load samples

Step 2.

Always load the gel with the reference sample. Chemoluminiscent signals in different blots cannot be compared/quantified

Run samples in SDS running buffer

Step 3.

Prepare 1 liter of 1X SDS solution from the Bio-Rad 10X Tris/Glycine/SDS. Run gel 5 min at 90V, followed by 260V for 27 min (for a 5% acrylamide gel)

REAGENTS

10x Tris/Glycine/SDS 1610732 by Bio-rad Laboratories

Activate gel for detection of proteins using the Stainfree program from the Chemidoc Gel imaging system / ImageLab

Step 4.

Using the protocol for Stainfree, activate for 1 min and detect using Intense Bands as automatic detection setup.

The size of the gel image and the one from chemiluminischece have to be identical. For mini-gels we use 8,6 x 6,6 cm.

SOFTWARE PACKAGE (Windows)

Image Lab Software, 5.2.1

Bio-rad laboratories

Transfer proteins from gel to PVDF membrane

Step 5.

Using the TransTurbo system (Link to published protocol), blot membranes to a PVDF membrane 0,2 µm.

REAGENTS

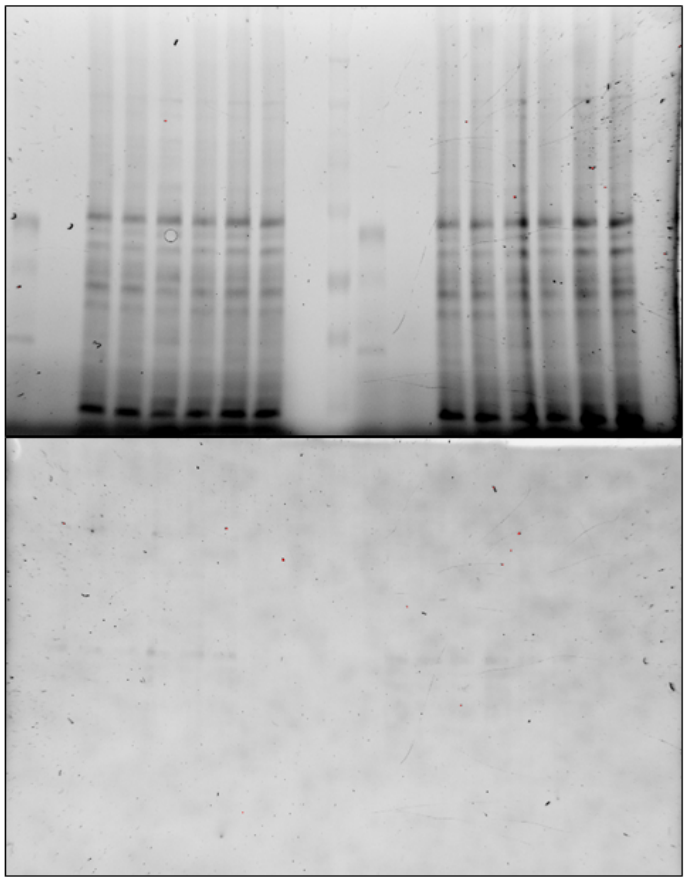
Immun-Blot® PVDF Membrane 0,2µm 1620177 by Bio-rad Laboratories

Verify efficiency of protein transfer

Step 6.

Once the transfer program is over, separate gel from membrane and place gel in destilled water. Dry the PVDF membrane for 1h at 37°C.

To verify the transfer efficiency, detect again the proteins left in the gel using the Stainfree probram but without activation and set the exposure time to exactly the same exposure time that the first detection used. The information can be found in the properties of the picture. Before evaluating the transfer efficiency, make sure that both picturers show the same range of grey levels (can be set in the Contrast tool). Transfer should be higher than 95%.



Detect proteins in membrane using Chemoluminicence or fluorescence detection

Step 7.

Use the normal protocol for westen blot to detect your protein.

for detection with chemoluminiscene using the ChemiDoc XRS+ System of Bio-rad laboratories. In the Image Lab software detect using the Chemi Hi Resolution with signal accumulation mode.

Save only images that have not reached saturation of detection.

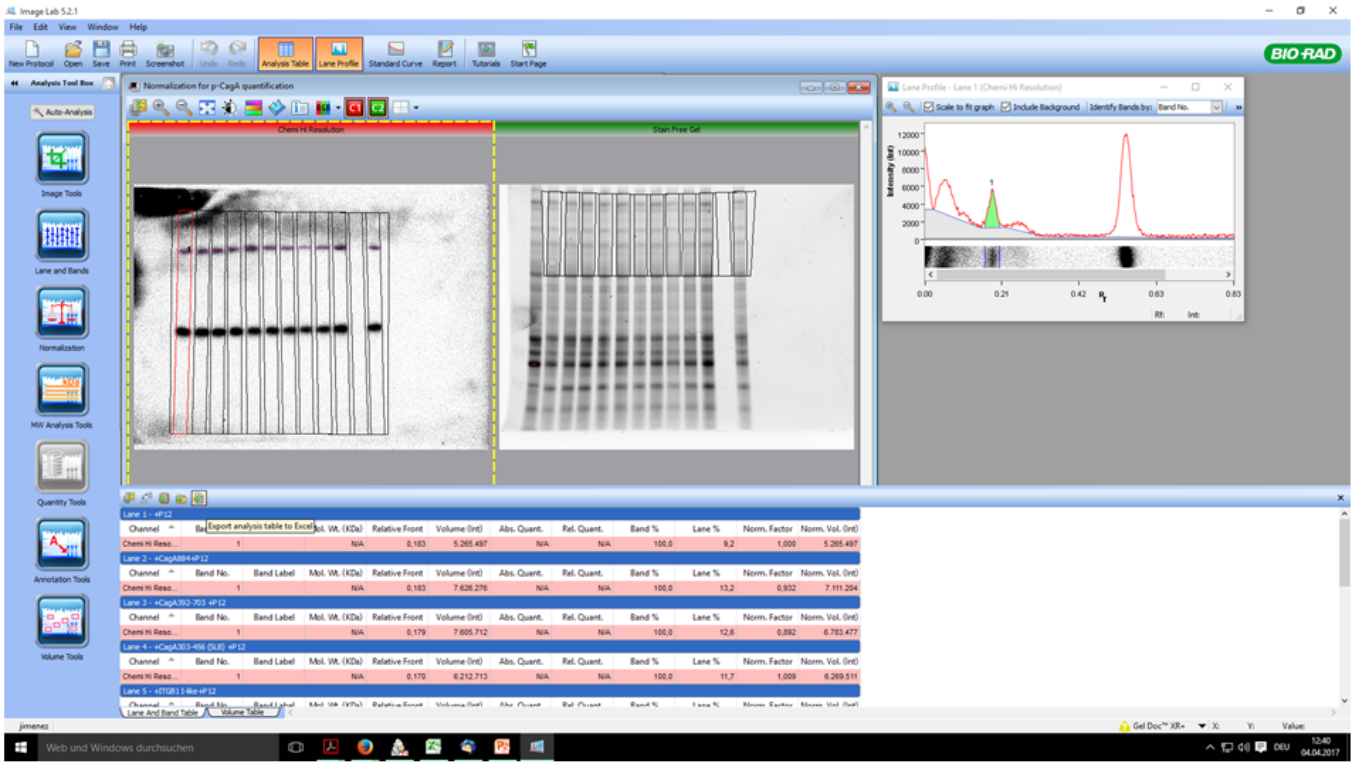
Use the normalization tool

Step 8.

Open both images: Chemi and Stainfree. Open Normalization tool and follow instructions. Once a multilayered image has been created, detect lanes on both images (Stainfree and Chemiluninscence) manually or automatic. Take care of markin all lanes, as similar as possible to reduce variations.

Mark bands corresponding to your protein. The automatic function of the software works great if the blot has no background. For verification or rectification of the selection, use the option 'Lane Profile'.

Once the bands are selected, open the 'Analysis Table' and export the information to an Excel file. This file will contain the normalization value relative to the first band selected. SAVE FILE IMAGE for documentation purposes



Quantify protein signals relative to control sample signal and normalize value

Step 9.

The quantification can be done using the Quantity tool. This option is NOT active in an multichannel image. For this reason, split the multichannel image. The Chemi Hi Resolution image will retain the bands. DO NOT CHANGE THEM. Select the Quantity tool and follow instructions to select the band reference.

Export data to excel and combine with normalization value: Normalization factor x Relative Quantity = Final value

Save the image for documentation purposes.

Store all values in a excel file

Step 10.

All data should be kept for later analysis and documentation purposes.

OPTIONALS

Step 11.

Normalization can be done as well using not the Total Protein in the lane, but using a reference band (Housekeeping protein, HKP). However this method is not recommended, since House Keeping Proteins amounts could have unknown variations depending on treatment.