NH DyeAGNOSTICS
STANDARDIZED PROTEIN ANALYSIS

Smart Protein Layers
Real-time Normalization of Western Blot Analysis

Jana Heise[1,2], Jan Heise[1]

[1] NH DyeAGNOSTICS GmbH, Weinbergweg, 23 06120 Halle, Germany
[2] Weinberg Laboratories, Weinbergweg 23, 06120 Halle, Germany

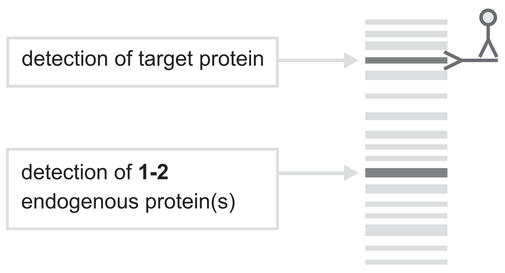
The Problem of Western Blot Normalization

Western blot analysis is a method to detect a target protein by immunochemistry in a complex sample.
The technique includes protein separation, its transfer to a membrane and diverse washing and antibody incubation steps.

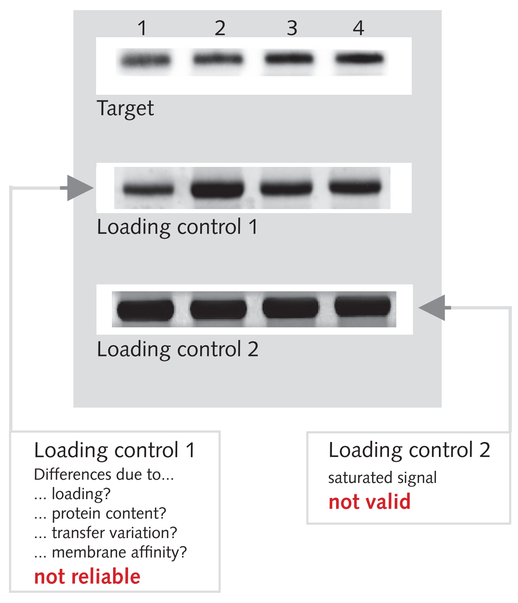
Reliable comparison of detected target signal intensities between different samples requires an appropriate way of normalization.

Reported fold changes of the target protein must not be based on an artifact of a reference signal (e.g. saturated pixel) and/ or experimental errors.

Principle



Immunochemistry



Normalization

Lane	1	2	3	4
Signal intensity px (AU)				
Target	1.890	2.230	3.480	3.610
Reference 1 (Loading control 1)	2.350	12.670	5.320	4.810
Reference 2 (Loading control 2)	< 65.000	< 65.000	< 65.000	<65.000
normalized Target / Ref. 1	**1,00**	**0,22 ?**	**0,81 ?**	**0,93 ?**
normalized Target / Ref. 2	**1,00**	**n.d.**	**n.d.**	**n.d.**

The Solution

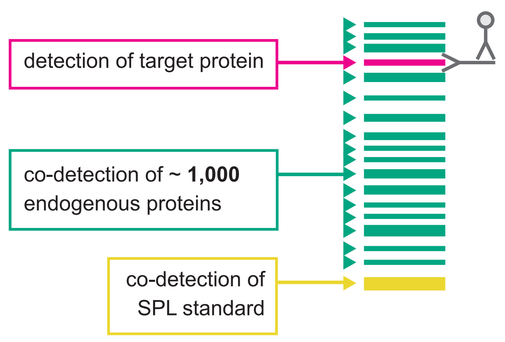
Smart Protein Layers (SPL) is an add-on kit for the detection of sample proteins present on the blot at the same time the target is immunodetected.

SPL is based on two components:

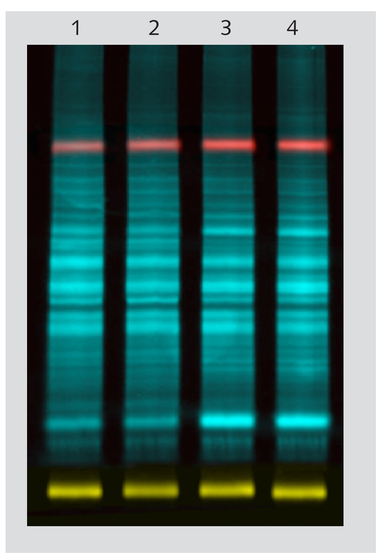
i) fluorescent SPL Labels bound to the sample protein prior separation,

ii) bi-fluorescent SPL Standards to monitor labeling efficiency, provide additional information about the sample, enable automated data evaluation.

Principle



Immunochemistry



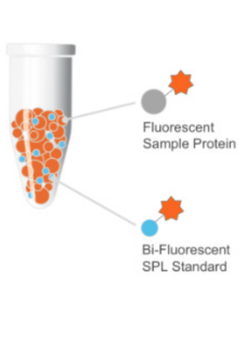
Normalization

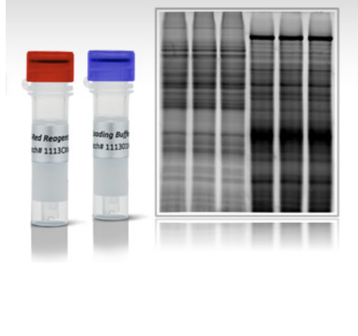
Lane	1	2	3	4
Signal intensity px (AU)				
Target	1.890	2.230	3.480	3.610
Reference real-time total	42.640	44.240	35.770	37.650
normalized Target	**1,00**	**1,14**	**1,54**	**1,67**

Conclusions

SPL offers

- real-time total protein detection in gels and blots
- normalization of the protein content between samples
- step-by-step monitoring of the complete Western Blot workflow
- precise normalization of the target protein expression in Western Blots
- accurate comparison of target protein expression between different experiments





SPL
real-time
gel & blot
monitoring