News: Smart Protein Layers

Real-time Normalization of Western Blots

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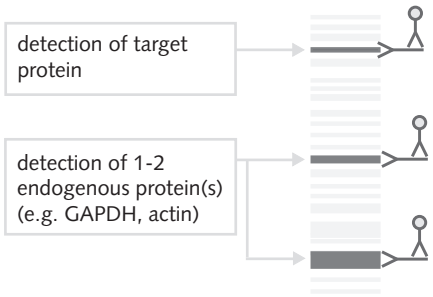
The Problem: Western Blot Normalization using internal Loading Controls/HKP

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.

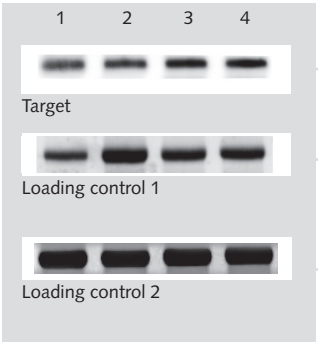
Reliable comparison of detected target signals between different samples requires appropriate normalization. Reported fold changes of the target protein must not be based on artifacts of a reference signal (e.g. saturation, regulation, experimental errors).

Target normalization based on "housekeeping"proteins (HPK) is not reliable. Various publications show that all HPKs are regulated. However, during WB procedere much protein is washed away from the membrane so reliable normalization must occur in real-time.

Principle



Immunodetection



Normalization

Detected target signal is normalized to 1 or 2 endogenous proteins (loading controls/ HPK)

Loading control 1: different signal intensities due to unequal loading or protein content or variations in transfer or membrane affinity etc.

Loading control 2: saturated signal resulting in invalid normalization

The Solution: Co-Detection of corresponding Total Protein for Normalization

Smart Protein Layers (SPL) is an add-on kit (patented technology). It allows the simple co-detection of sample total protein at the same time the target is immunodetected.

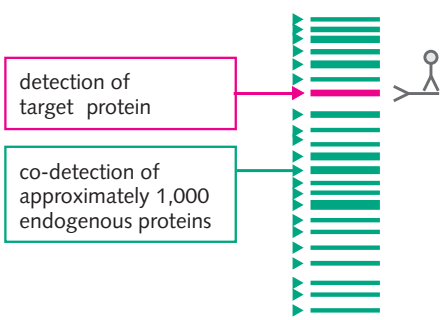
SPL is based on fluorescent SPL label rapidly bound to the sample protein prior electrophoresis.
Depending on sample properties two different workflows can be used.

The **SPL easy workflow** allows for the normalization of target to total protein for samples within a range of 25µg - 75µg of extracted total protein.

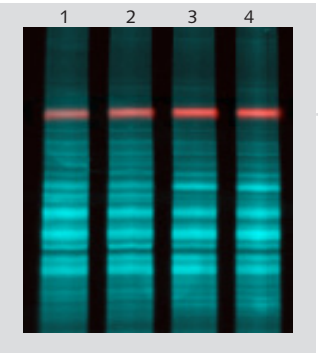
The **SPL advanced workfow** allows for normalization of target to total protein for scarce samples and or particular buffer compositions. This approach provides further information about the sample, the labeling reaction and monitors the complex WB workflow.

Both the easy and advanced workflow workflows offer precise protein transfer monitoring and allow for experiment-to-experiment comparisons.

Principle



Immunodetection target + co-detection total protein



Normalization

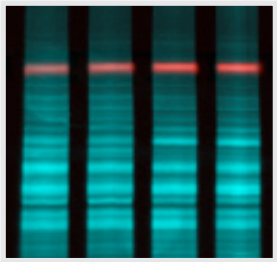
Detected target signal is normalized to all sample proteins present on the blot at this time.

Smart Protein Layers

Easy workflow for total protein detection in Western Blots

The **SPL easy workflow** allows for the normalization of target to total protein for samples within a range of 25µg - 75µg of extracted total protein.

Total protein is pre-labeled with SPL fluorophores prior gel electrophoresis (fig.1). At time the target is immuno-detected the amount of present sample on the blot is sensitively determined as well (fig.2). This allows to precisely normalize the obtained target signal in ratio to its total protein present on the blot at this point of time.



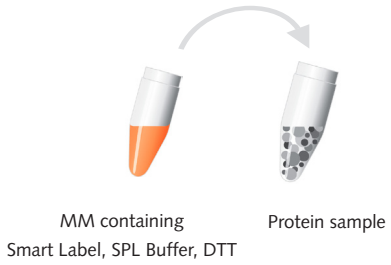


Fig. 1. Sample preparation for total protein SPL labeling.

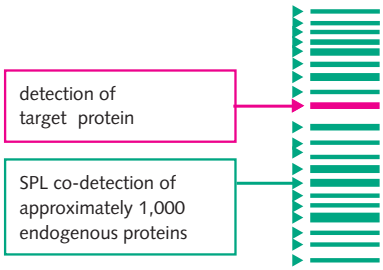


Fig. 2. Detection of target + co-detection of corresponding sample.

Key feature	**fast & easy detection of total protein & normalization of target protein signal**
Protein sample requirements*	
amount of protein per well	25 to 75 µg (determination of protein concentration is required)
compatible buffer ingredients	salts, buffering agents (e.g. Tris, HEPES, MOPS, etc.), detergents (ionic and non-ionic), chelators, protease inhibitors; amines (≤100 mM) and reducing agents (≤1 mM DTT; ≤1% ß-mercaptoethanol) e.g. standard buffers like RIPA buffer, NP-40 or SDS buffer
Workflow	• add MM^{easy} containing SPL buffer + SPL label + DTT to sample • incubate at 95°C for 5 min • perform electrophoresis • (opt) check for separation quality by rapid imaging • transfer protein to membrane • (opt.) check for protein transfer quality • perform immunochemistry (blocking, washing, 1st/2nd AB) as ususal • detect target and corresponding total protein • normalize target signal to its total protein
Additional features	• kit includes fluorescent MW marker (12.5 kDa, 25 kDa, 80 kDa) • monitoring of secondary antibody performance • comparison of different WB experiments

* > 75 µg: dilute or switch to advanced WF; < 25 µg: use advanced WF; other buffer composition: use advanced WF

Smart Protein Layers

Advanced workflow for total protein detection in Western Blots for comprehensive analysis, scarce proteins, and special samples

The **SPL advanced workfow** allows for normalization of target to total protein for scarce samples and/or particular buffer compositions. In addition, the advanced workflow provides further information about the sample, the labeling reaction and monitors the complex and sometimes error-prone Western Blot procedure.

Total protein and the SPL standard (SMA) are pre-labeled with SPL fluorophores prior gel electrophoresis (fig.1). At time the target is immuno-detected the amount of present sample on the blot is sensitively determined as well (fig.2). This allows to precisely normalize the obtained target signal in ratio to its total protein present on the blot at this point of time.

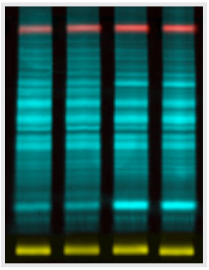


Fig. 1. Sample preparation for total protein SPL labeling incl. SMAs.

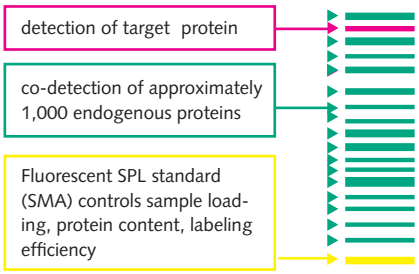


Fig. 2. Detection of target protein + co-detection of corresponding sample protein + SPL standard.

Key feature	**comprehensive normalization of total protein & target protein signal**
Protein sample requirements	
amount of protein per well	0,5 to 100 µg (exact determination of protein concentration is not required)
compatible buffer ingredients	salts, buffering agents (e.g. Tris, HEPES, MOPS, etc.), detergents (ionic and non-ionic), chelators, protease inhibitors; amines (≤400 mM) and reducing agents (≤5 mM DTT; ≤1% ß-mercaptoethanol) eg. standard buffers like RIPA Buffer, NP-40 or SDS Buffer
Workflow	• add MM advanced containing SPL buffer + SMA + DTT to sample • add Smart Label and incubate at 95°C for 5 min • perform electrophoresis • detect SMA and total protein (required) • transfer protein to membrane • (opt.) check for protein transfer quality • perform immunochemistry (blocking, washing, 1st/2nd AB) as ususal • detect target and corresponding total protein • normalize target signal to its total protein
Additional features	• fluorescent MW marker (12.5 kDa, 25 kDa and 80 kDa) is included • monitors sample application onto the gel • monitors labeling efficiency • monitors protein transfer onto membrane • monitors performance of secondary antibody • compares different experiments

Smart Protein Layers

Kit selection

Target detection	Recommended SPL Kit (label of total protein)	Workflow *	Required imaging properties	Product no.
HRP-conjugated secondary antibody (for ECL)	SPL Red (red labeled total protein)	easy workflow	Chemiluminescence & red fluorescence	SPL Red Kit 200 rcts. (20W): PR913 400 rcts. (40W): PR926
		advanced workflow	Chemiluminescence, blue & red fluorescence	
green fluorescent secondary antibody	SPL Blue or Red (blue labeled respec. red labeled total protein)	easy workflow	blue or red & green fluorescence	SPL Blue Kit 200 rcts. (20W): PR916 400 rcts. (40W): PR925 SPL Red Kit 200 rcts. (20W): PR913 400 rcts. (40W): PR926
		advanced workflow	blue, green and red fluorescence	
red fluorescent secondary antibody	SPL Blue (blue labeled total protein)	easy workflow	blue & red fluorescence	SPL Blue Kit 200 rcts. (20W): PR916 400 rcts. (40W): PR925
		advanced workflow	blue & red fluorescence	
infrared fluorescent secondary antibody	SPL Red-IR (red labeled total protein)	easy workflow	red & infrared fluorescence	SPL Red-IR Kit 200 rcts. (20W): PR917 400 rcts. (40W): PR927
		advanced workflow	red & infrared fluorescence	

* use SPL easy workflow when 25 to 75 µg protein amount per well and ordinary buffer composition. Use the SPL advanced workflow for scarce samples or special buffer composition (for detailed information see SPL kit product guide).

Related products

Fluorescent and HRP-conjugated Seconadaries	Description	Size	Product no.
green	Smart Green Fluorescent Anti-Mouse anti-mouse IgG from goat, affinity purified	0,5 mg	PR835
	Smart Green Fluorescent Anti-Rabbit anti-rabbit IgG from goat, affinity purified	0,5 mg	PR841
	Smart Green Fluorescent Anti-Goat anti-goat IgG from rabbit, affinity purified	0,5 mg	PR833
red	Smart Red Fluorescent Anti-Mouse anti-mouse IgG from goat, affinity purified	0,5 mg	PR832-M
	Smart Red Fluorescent Anti-Rabbit anti-rabbit IgG from goat, affinity purified	0,5 mg	PR832-R
	Smart Red Fluorescent Anti-Goat anti-goat IgG from rabbit, affinity purified	0,5 mg	PR832-G
infrared	Smart IR Fluorescent Anti-Mouse anti-mouse IgG from goat, affinity purified	0,5 mg	PR838
	Smart IR Fluorescent Anti-Rabbit anti-rabbit IgG from goat, affinity purified	0,5 mg	PR837
	Smart IR Fluorescent Anti-Goat anti-goat IgG from rabbit, affinity purified	0,5 mg	PR843
blue	Immuno Blue HRP-Substrate	100ml for ca. 400 rcts.	PR840