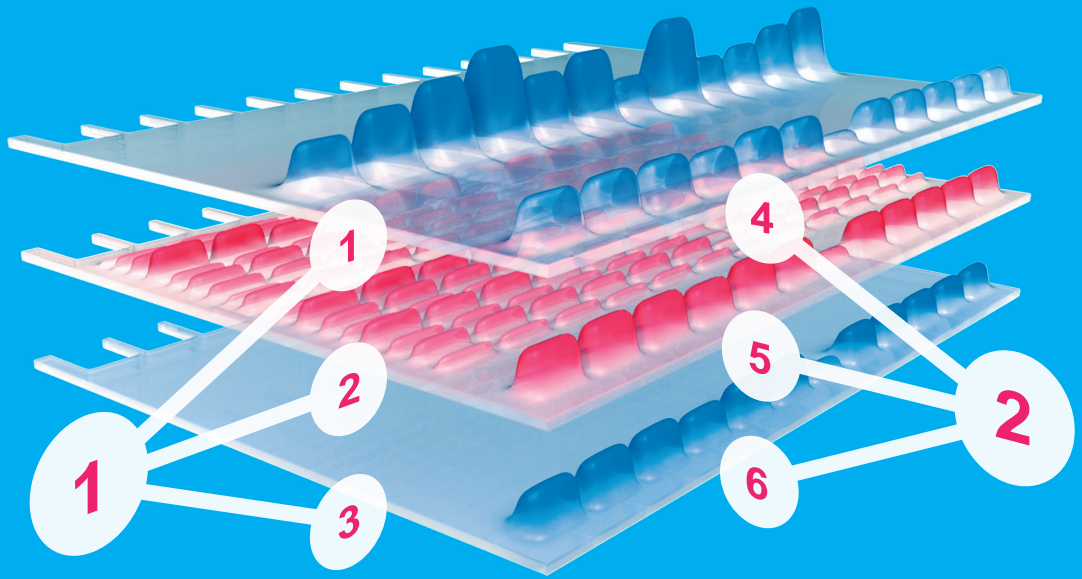
NH DyeAGNOSTICS

SMART PROTEIN LAYERS

Quantitative and standardized protein gel and Western blot analysis



The challenge

There is an increasing demand for quantitative, sensitive and reliable 1D gel and Western blot (WB) analyses. However, standardization, normalization and precise quantification of different protein samples separated by 1D gel electrophoresis is difficult due for various reasons. Visualization of proteins using stains like *Coomassie Blue* for gels or *Ponceau Red* for blots suffers from low sensitivity, limited dynamic range and poor reproducibility.

Obtaining quantitative data from WB requires an appropriate method of normalization to assure that the reported fold changes of the target protein are not an artifact of reference signal and experimental errors*.

A typical approach is to use signal normalization of target based on housekeeping proteins (HKPs) like ß-actin, ß-tubulin or GAPDH. However, as it becomes clear that HKPs are differing in their abundance under various conditions (e.g. stress, treatments), this approach may no longer be suitable.
The use of total protein transferred to the blot is a more accurate source of data normalization**. As proteins are washed away from the membrane during WB procedure, target and reference proteins should be detected at the same moment to achieve accurate quantification.

* Taylor and Posch. 2014. The design of a quantitative Western blot experiment. BioMed Research International. Article ID 361590

** Colella et al. 2012. Comparison of stain-free gels with traditional immunoblot loading control. Anal. Biochem. 430 (2): 108-110

** Guertler et al. 2013. Stain-free technology as normalization tool in western blot analysis. Anal. Biochem. 433 (2): 105-111

The solution

Smart Protein Layers (SPL) is the new technology for stain-free, quantitative and standardized analysis of protein gels and Western blots. For the first time the amount of target protein and the amount of total protein of the sample (as a reliable reference) present at this moment can be detected. In addition, SPL allows to precisely compare and quantify data derived from different experiments.

SPL offers:

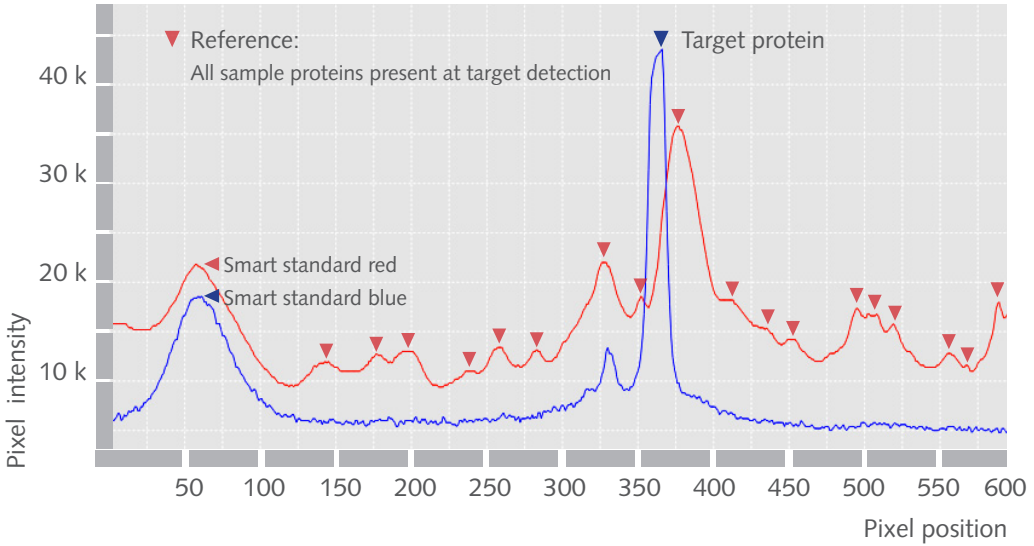
- Stain-free, very sensitive protein visualization on gels and blots
- Protein content equalizer (loading by volume, inaccurate protein determination, loss of sample)
- Monitoring of every step during the Western blot analysis
- Accurate quantification of target protein expression
- Precise comparison of different experiments

The SPL technology is based on fluorescent label for total protein detection combined with a sample-dependent bi-fluorescent standard. This combination allows for rapid and highly sensitive protein detection with a new quality of standardization and accurate quantification of expression levels within one experiment and between different experiments.

Smart Protein Layers

The first standard-based technique for quantitative Western blots.

Band profile of a SPL total protein lane at the moment of target detection



Smart Protein Layers

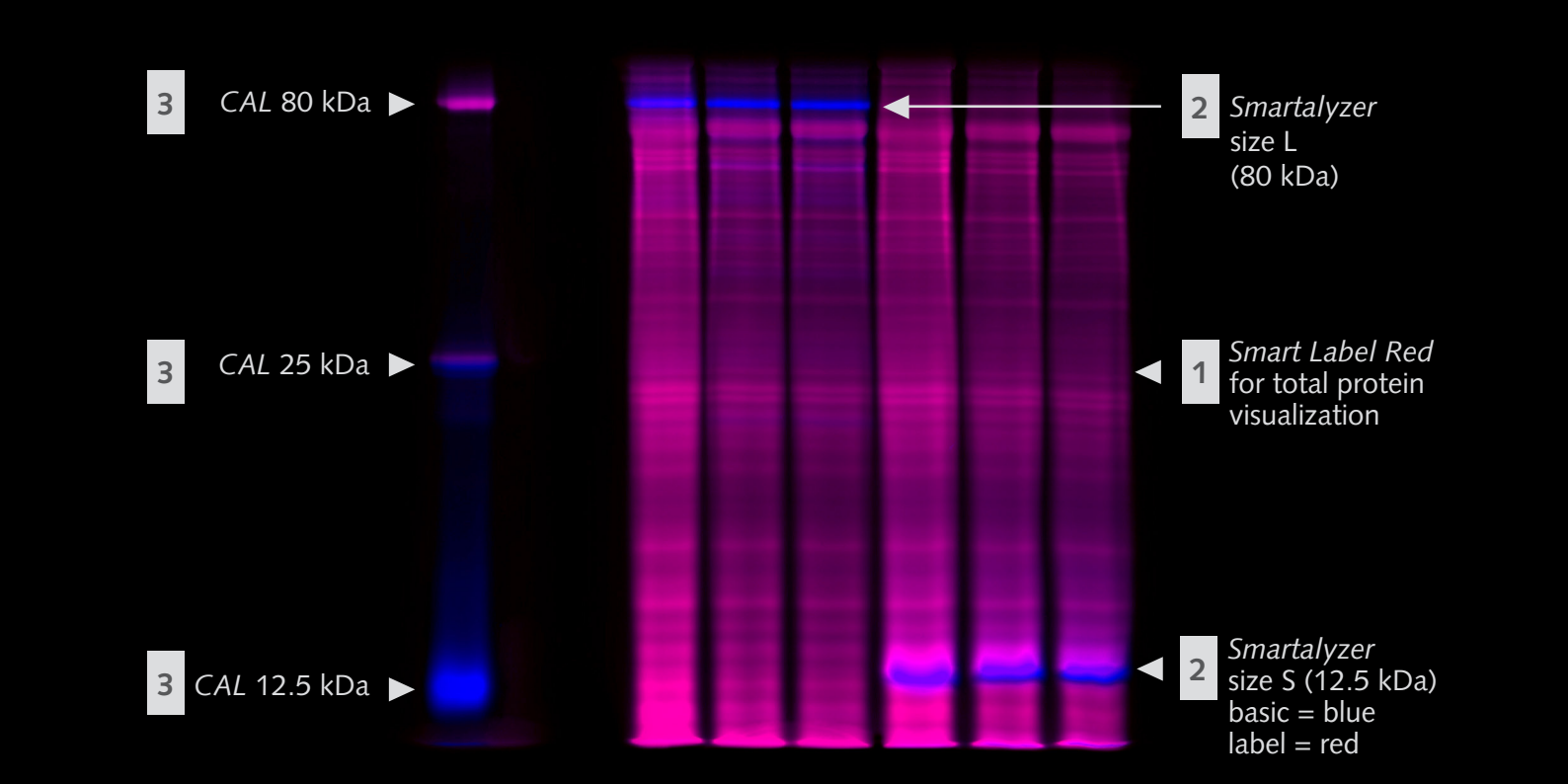
Quantitative and standardized protein gel and Western blot analysis

Smart Protein Layers (SPL) is based on three components:

1. The fluorescent *Smart Label* visualizes total protein in the gel and on the blot with high sensitivity (detection limit less than 1 ng, dynamic range 10^4 - 10^5) within seconds. *Smart Labels* are chemically bound to the protein prior protein separation (3 min hands-on).

2. The bi-fluorescent *Smartalyzer* (*SMA*) is a multi-functional standard added to every sample prior to separation. For precise normalization, standardization and quantification of total protein.
SMA is available in size S (12.5 kDa) and size L (80 kDa).

3. The bi-fluorescent *Calibrator* (*CAL*) is the standard for comparison protein expression between different gels or blots. *CAL* also works as bi-fluorescent molecular weight marker (80 kDa; 25 kDa; 12.5 kDa).

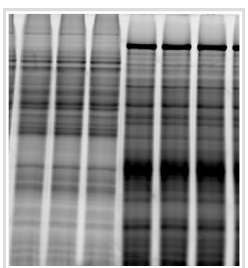

3 CAL 80 kDa
3 CAL 25 kDa
3 CAL 12.5 kDa
2 Smartalyzer size L (80 kDa)
1 Smart Label Red for total protein visualization
2 Smartalyzer size S (12.5 kDa) basic = blue label = red

The Smart Label

Stain-free and sensitive protein visualization of gels and blots

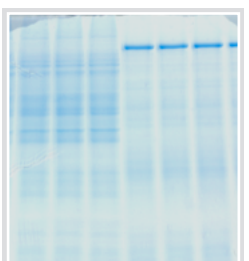
The fluorescent *Smart Label* visualizes total protein in the gel and on the blot with high sensitivity (detection limit less than 1 ng, dynamic range 10^4 - 10^5) within seconds. *Smart Labels* are chemically bound to the protein prior protein separation (3 min hands-on).

Smart Label



20 µg protein / lane

Coomassie stain



20 µg protein / lane

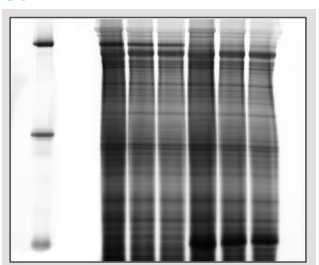
	Coomassie stain	Fluorescent stain	Smart Label
Detection limit (ng)	100 - 10	1 or less	1 or less
Dynamic range	E2	E4-5	E 4-5
Staining/ destaining	yes	yes	not required
Reproducibility	low	low	very high
Protein monitoring	no	no	yes

The Smart Label

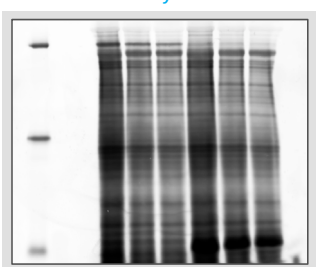
Monitoring total protein during every step of the Western procedure

Protein transfer and subsequent Western blot procedure (e.g. washing steps with TBST) lead to inconsistent loss of total protein. The high sensitivity *Smart Label* precisely monitors the amount of protein for every protein band at every step of the Western blot procedure. Figures show sample protein (20 µg per lane) visualized by using *Smart Labels* during Western blot analysis. Rapid protein detection was performed using an Octoplus Fluorescence Imager.

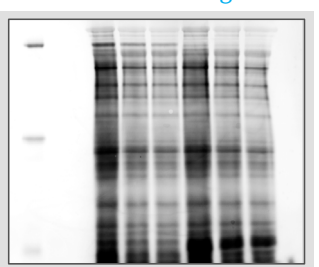
Gel



Blot immediately after transfer



Blot at moment of target detection

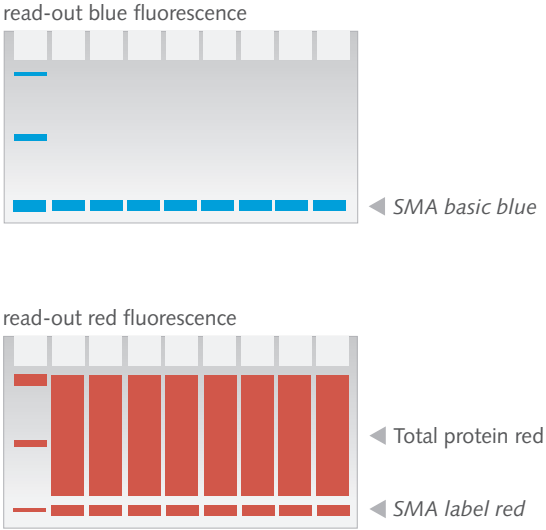


The Smartalyzer

Using a smart standard for accurate normalization

Providing standards to samples allows for precise normalization. The *Smartalyzer* (*SMA*) is a blue fluorescent standard which is added to every protein sample. The sample and the *SMA* are then labeled with reactive *Smart Label Red*.

After this procedure the sample protein fluoresces in red, *SMA* fluoresces in basic blue and in labeling red. *SMA* is available in two sizes: *SMA* S = 12.5 kDa, *SMA* L = 80 kDa.

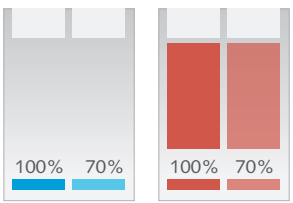


The Smartalyzer

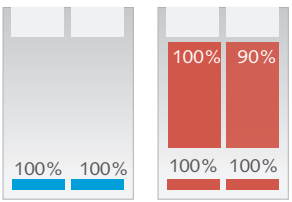
Using a smart standard for accurate normalization

The relation between the blue and red fluorescent *SMA* and the corresponding red fluorescent sample protein allows for precise lane to lane protein normalization. This includes normalization of unequally loaded lanes due to loss of sample (figure A) or samples differing in their protein content (figure B). The labeling efficiency is monitored and if necessary normalized (figure C).

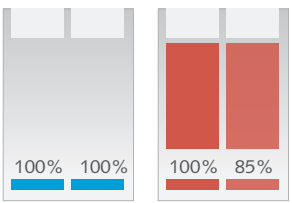
A Use *SMA basic blue* for loading normalization.



B Use protein label red for normalization of samples loaded by volume or incorrect protein determination.



C Use *SMA label red* for labeling efficiency normalization.

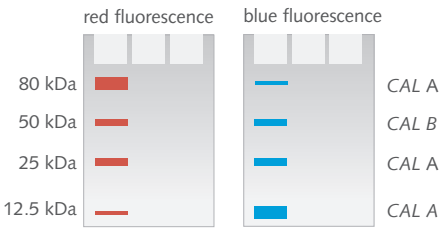


The Calibrator

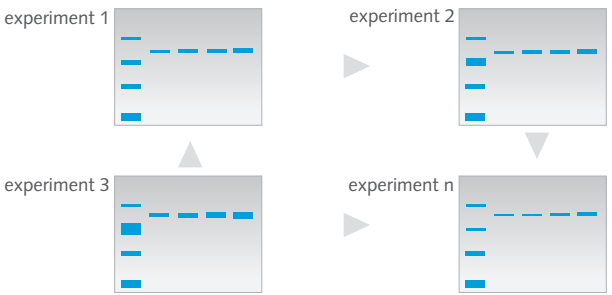
The standard for experiment-to-experiment comparison

For comparison of different experiments, a bi-fluorescent standard, the *Calibrator* (*CAL*) is applied to the gel. *CAL* consists out of three markers (*CAL A*, each red and blue fluorescent) and one 50 kDa marker (*CAL B*) specific to the 2nd antibody used for these experiments.
In addition, *CAL* is a fluorescent protein weight marker.

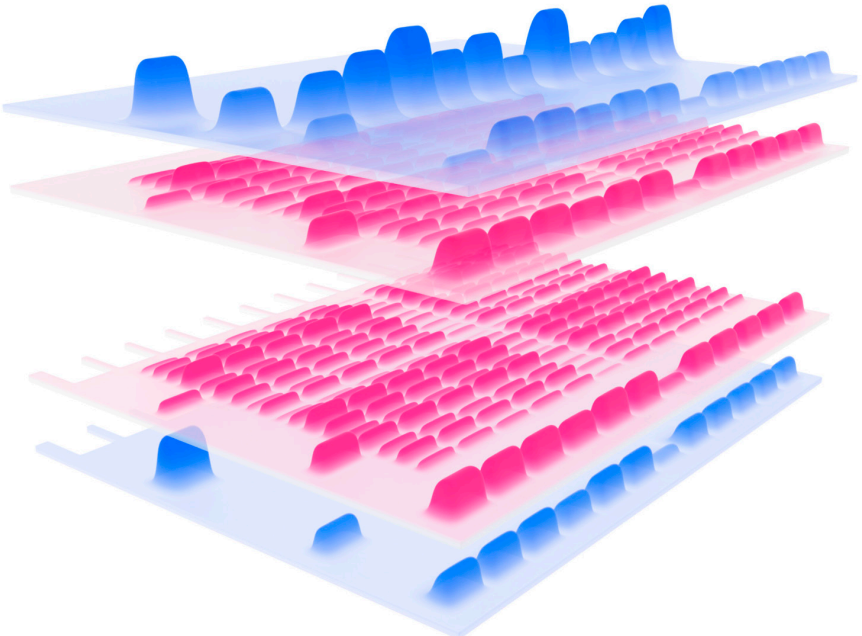
Use *CAL A* as fluorescent marker



Use *CAL A+B* to compare different gels or blots

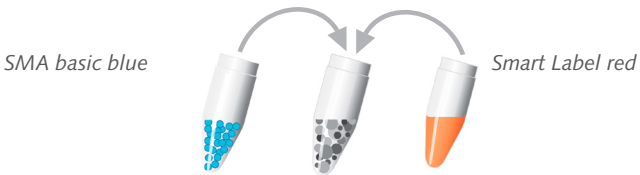


Smart Protein Layers
Layer overview



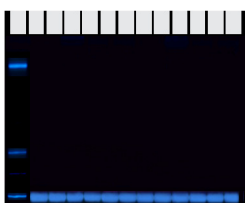
Smart Protein Layers Workflow

1 Add *Smart Label* & *Smartalyzer* (*SMA*) to your protein.

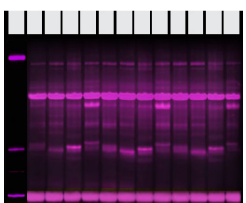


2 Run SDS-PAGE. Detect blue and red fluorescence.

- *SMA basic blue*
- *CAL A*



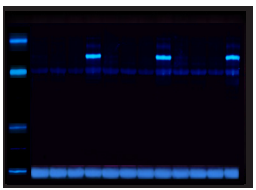
- Total protein
- *SMA label red*
- *CAL A*



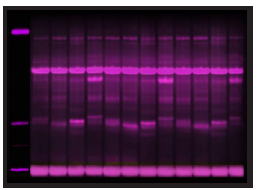
Smart Protein Layers Workflow

3 Perform Western blot and detect fluorescence of target (blue) and normalized total protein

- *SMA basic blue*
- *CAL A + B*

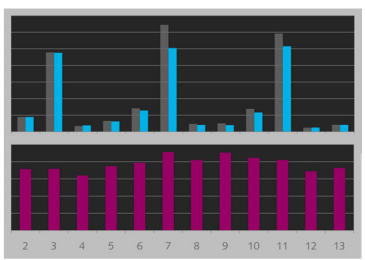


- Total protein
- *SMA label red*
- *CAL A*



4 Process data and evaluate.

- Detection and determination of band and lane raw volumes.
- Normalization of gel load (based on *SMA basic blue*).
- Normalization of *Smart Label* (based on *SMA label*).
- Normalization of target protein signal (based on total protein).
- Experiment-to-experiment normalization (based on *CAL*).

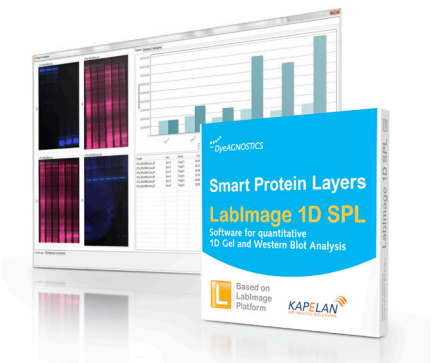


SPL-LabImage Software

Fast and accurate data processing and evaluation

Accurate evaluation requires several data processing steps. The SPL-LabImage software is designed to determine band and lane raw volumes of an unlimited number of projects. It automatically uses *SMA basic color*, *SMA label color* and protein signal to perfectly normalize the total protein of gels and blots. Normalization of target protein expression can then be precisely performed.

- Workflow-based software
- Maximum flexibility (e.g. reference lane can be freely chosen)
- Runs on MAC or PC (English or German)



Target detection

Which SPL kit fits to my method of target protein detection?

The best method (in terms of signal stability, easy handling, exposure time) of your target protein detection depends on target abundance in the sample. Higher abundant proteins can be easily detected using first or secondary fluorescent antibodies. For most targets detection using Immuno blue is the ideal choice. However, very low abundant protein targets (femtogram range) can be detected only be using adequate femto chemiluminescence kits. The table below shows recommended combinations of SPL kits for total protein visualization and the optimal method for target protein detection.

Target protein	Detection method	SPL kit
higher picogram range	fluorescent antibody conjugates (preferably red or infra red)	SPL Blue
lower picogram range	Immuno Blue fluorescence + HRP conjugated 2nd AB	SPL Red
femtogram range	chemiluminescence kits for femtogram range	SPL Red

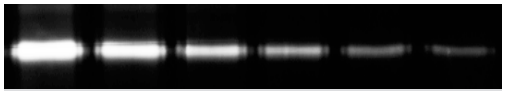
Immuno Blue Fluorescent Substrate

Target protein detection

Immuno Blue Fluorescent Substrate combines the high sensitivity of chemiluminescence with signal stability and the short exposure time of fluorescent antibody conjugates.

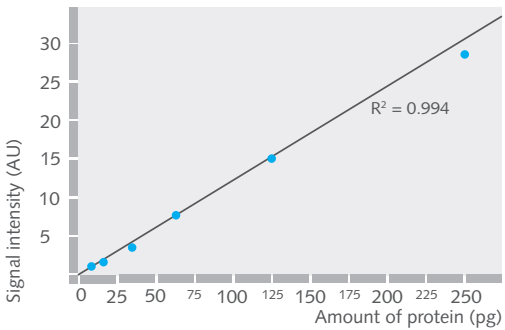
HRP-conjugated 2nd antibodies transform Immuno Blue into a fluorescent compound which precipitates and remains stable on the blot.

- Short exposure time (10 -100 x shorter than ECL)
- Long signal stability of several months
- Sensitivity in the lower pg range



250 125 62 31 16 8

pg of detected target protein (casein) by Immuno Blue and HRP-conjugated 2nd antibody. Exp. time: 15 sec



Fluorescence imaging requirements

Smart Protein Layers can be easily read-out with epi-blue (496/ 520 nm) and epi-red (650/ 665 nm) fluorescence imagers.

The following options are recommended:

- Red + blue fluorescence available:
  Choose SPL Red for total protein + Immuno Blue for target protein detection
  Choose SPL Blue for total protein + Smart Red 2nd AB for a highly abundant target
- Blue fluorescence + chemiluminescence available:
  Choose SPL Blue for total protein, chemiluminescence for target protein detection

For further questions please contact info@dyeagnostics.com

Product overview

SPL Red for 40 Western blots

recommended in combination with Immuno Blue (PR 840)

- *Smart Label red*
  400 reactions for 1-20 µg of protein each
- *Smartalyzer basic blue*
  choose size S (12.5 kDa) or size L (80 kDa)
- *Calibrator red & blue*
  3 fluorescent marker bands, choose *Calibrator B* specific to 2nd antibody

Kit description	Smart Label	Smartalyzer	Calibrator	Product No.
SPL Red S Mouse	red	SMA basic blue S	Mouse	PR911-M
SPL Red S Rabbit	red	SMA basic blue S	Rabbit	PR911-R
SPL Red S Goat	red	SMA basic blue S	Goat	PR911-G
SPL Red L Mouse	red	SMA basic blue L	Mouse	PR912-M
SPL Red L Rabbit	red	SMA basic blue L	Rabbit	PR912-R
SPL Red L Goat	red	SMA basic blue L	Goat	PR912-G

Product overview

SPL Blue for 40 Western blots

recommended in combination with Smart Red fluorescent 2nd antibody

- *Smart Label blue*
  400 reactions for 1-20 µg of protein each
- *Smartalyzer basic red*
  choose size S (12.5 kDa) or size L (80 kDa)
- *Calibrator red & blue*
  3 fluorescent marker bands, choose *Calibrator B* specific to 2nd antibody

Kit description	Smart Label	Smartalyzer	Calibrator	Product No.
SPL Blue S Mouse	blue	SMA basic red S	Mouse	PR914-M
SPL Blue S Rabbit	blue	SMA basic red S	Rabbit	PR914-R
SPL Blue S Goat	blue	SMA basic red S	Goat	PR914-G
SPL Blue L Mouse	blue	SMA basic red L	Mouse	PR915-M
SPL Blue L Rabbit	blue	SMA basic red L	Rabbit	PR915-R
SPL Blue L Goat	blue	SMA basic red L	Goat	PR915-G

Product overview

Immuno Blue Fluorescent Substrate
for 40 Western blots 10 x 10 cm
Product No. PR 840

Smart Red fluorescent 2nd Antibody
0.5 mg
Product No. PR 831 (for anti-mouse)
Product No. PR 832 (for anti-rabbit)
Product No. PR 832-G (for anti-goat)

Blotting Kit
low fluorescent membrane + blotting paper,
precut 10 x 10 cm, pack of 10
Product No. PR 811

Beo Dry Blotter
high qualitity blotting of up to 18 mini gels, no buffers required
Product No. PR87

SPL-LabImage Software
for quantitative 1D gel and Western blot analyses
Mac or PC, English or German version
Product No. PR 989

Octoplus SPL Imager
red and blue fluorescence (optional: + green, + IR)
high end chemiluminescence detection


DyeAGNOSTICS
Smart Protein Layers
LabImage 1D SPL
Software for quantitative
1D Gel and Western Blot Analysis
Based on
LabImage
Platform
KAPELAN
SPL Red
Smart Protein Layers
DyeAGNOSTICS