

## Produktinformation

# Smart Protein Layers Data Processing & Evaluation

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## 1 Content

This product guide describes the processing and evaluation of quantitative gel and Western blot analyses by using the Smart Protein Layers technology. Target protein expression can be analyzed precisely by using the appropriate reference that is SPL normalized total protein.

## 2 Required software

The detection and determination of band volumes and lanes in 1D gels and on Western blots requires an appropriate software.

We recommend to use the SPL-LabImage software (Prod. No. PR 989) as it allows for fast and easy data processing and evaluation including automatization of all normalization steps.

For starters a free basic version, the LabImage 1D L300 for PC (32 bit), PC (64 bit) or Mac (64 bit) is available. It allows for band and lane detection of gels and blots. However, acquired data must be analyzed further using additional software (e.g. EXCEL).

Klick [here](http://www.kapelan-bioimaging.com/software/platform/download-software.html) for a free download oder goto:  
<http://www.kapelan-bioimaging.com/software/platform/download-software.html>

## 3 Data Processing & Evaluation

### Step 1 Nomenclature of the acquired images

The SPL analysis requires 4 fluorescence images. These are:

- Total protein (incl. SMA label) after gel electrophoresis (GTO = gel total protein)
- SMA basic after gel electrophoresis (GLO = gel loading control)
- Total protein after western blot analysis (BTO = blot total protein)
- Target protein after western blot analysis (BTA = blot target protein)

We recommend to indicate the acquired image files as GTO, GLO, BTO and BTA, respectively and to add a tag for the experiment (e.g. date). Separate abbreviation, experiment tag and other information by underscores:

e.g.: GTO\_Experiment1\_April15\_2014.tif

*Note: Only this nomenclature allows for an automated normalization of target protein signal using SPL-LabImage software.*

### Step 2 Detection of lanes and bands

Detect lanes and bands of your gel and blot images using appropriate software. The following data are required for the SPL-analysis:

- Band volume of SMA basic detected in GLO
- Band volume of SMA label detected in GTO
- Lane volume (without band volume of SMA label) detected in BTO
- Band volume target protein detected in BTA
- for experiment-to-experiment comparisons: band volume of CAL of the distinct experiments

### Step 3 Normalization of signal of the target protein

When using SPL-LabImage 1D software, all normalizations steps will be processed automatically. SPL-LabImage 1D provides you the normalized band volumes and distinct normalization factors (SPL factor). When using a different software, follow this guide to accurately normalize your target signals.

### 1. Normalization of protein load (based on SMA basic):

If equal amounts of the standard (SMA) have been added to every sample, differences in the amount of SMA basic (see image GLO) are caused by a loss of sample during sample preparation or loading. To adjust use the normalization factor for load (**NF Load**).

Choose one lane / protein sample as your reference lane.

Determine **NF Load** in GLO:

$$\text{NF Load lane X} = \frac{\text{band volume SMA basic of lane X}}{\text{band volume SMA basic of reference lane}}$$

The **NF Load** now allows for the normalization of:

band volumes **SMA label** in GTO  
lane volume total protein in BTO  
band volume of target protein signal in BTA.

$$\text{normalized lane (respectively band) volume of lane X} = \frac{\text{lane (band) volume of lane X}}{\text{NF Load of lane X}}$$

### 2. Normalization of the Smart Label

SMA basic is added to every protein sample and both, SMA and sample are labeled under identical conditions. Differences in the band volume of the SMA label (in GTO) display differences in the labeling efficiency of the samples.

Determine the normalization factors for the labeling (**NF Label**) of the different lanes (= different protein samples) in GTO. Choose one lane (= one sample) as reference.

Determination of **NF Label** in GTO:

$$\text{NF Label lane X} = \frac{\text{band volume SMA label of lane X}}{\text{band volume of SMA label of reference lane}}$$

Use **NF Label** for the normalization of:

lane volume total protein in BTO.  
normalized lane volume of lane X =  
$$\frac{\text{lane volume of lane X}}{\text{NF Label of lane X}}$$

*Note: Normalize SMA label with NF Load before determination of NF Label. Do not normalize target protein signal with NF Label!*

### 3. Normalization of the target signals

Differences in the amount of protein of the different samples (due to missing or wrong determination of the protein content) will result in variations of target protein signals. In order to avoid misinterpretations, normalization of target protein to total protein is required. Choose a reference lane and determine the normalization factor **NF Total** in BTO.

Determination of **NF Total** in BTO:

$$\text{NF Total lane X} = \frac{\text{lane volume (excl. SMA label) of lane X}}{\text{lane volume (excl. SMA label) of reference lane}}$$

**NF Total** now allows for the normalization of:

Target protein signals in BTA.

$$\text{normalized band volume of target protein lane X} = \frac{\text{band volume of target protein lane X}}{\text{NF Total lane X}}$$

## Step 4 Experiment-to-experiment comparisons

In order to compare fluorescence and/ or target protein signals derived from different gels or blots a point of origin is required. This is given by the Calibrator (CAL). Choose one gel or one blot as your reference. Use for comparisons of different experiments always the same out of the three possible fluorescent CAL A bands. Choose such a CAL A band which can be visualized/ detected in all of your experiments and which is below signal saturation.

For each fluorescence channel the factor of normalization of CAL (NF CAL) can be determined by:

$$\text{NF CAL experiment X} = \frac{\text{band volume CAL of experiment X}}{\text{band volume CAL of reference experiment}}$$

The generated NF CAL now serves for the normalization of signals derived from different experiments:

$$\text{Normalized band volume of experiment X (lanes Y to Z)} = \frac{\text{band volume target of experiment X (lanes Y to Z)}}{\text{NF CAL experiment X}}$$

*Note: When using different lots of CAL the lot-dependent CAL factor must be taken into account. CAL-normalized band volumes of the target proteins must be divided additionally by this factor.*