NH DyeAGNOSTICS

Product Guide

Smart Protein Layers SPL Gel Kit Red

Product no. PR901, PR902

NH DyeAGNOSTICS GmbH
Weinbergweg 23
D-06120 Halle

Technical Support
Fon: +49 (0) 345-27 99 64 13
e-mail: service@dyeagnostics.com
www.dyeagnostics.com

FOR RESEARCH USE ONLY

1 Products and content

	SPL Gel Kit Red 20G	SPL Gel Kit Red 40G
Product no.	PR901	PR902
Smart Label Red Reagent A*	1x 200 Rcts	2x 200 Rcts
Smart Label Red Reagent B*	1x 200 Rcts	2x 200 Rcts
SPL Buffer	1x	2x
SPL Smartalyzer basic blue size S (SMA; 12,5 kDa)	1x	2x
SPL Smartalyzer basic blue size L (SMA; 80 kDa)	1x	2x
SPL Calibrator A (CAL A)**	1x	2x

* sufficient for 20 or 2x20 Minigels / 200 or 2x200 Smart Label reactions of up to 100 µg protein per sample
** for experiment-to-experiment comparisons

2 Storage and stability

Store all kit components at -20°C to -80°C
Store the Smart Label Working Solution at -20°C to -80°C

Best before: see kit packaging
The Smart Label working solution is stable for up to 6 month.

3 Safety instructions

The product should only be used by appropriately trained personnel. Basic laboratory safety standards must be observed during use. Adequate protective clothing such as a lab coat, gloves and protective goggles must be provided.

Smart Label Reagent B contains dimethylformamide (DMF; CAS number 68-12-2; H-phrases: H360D - H226 - H332 - H312 - H319; P-phrases: P201 - P210 - P302 + P340 - P305 + P351 + P338 - P308 + P313). DMF is harmful if inhaled, swallowed or in contact with the skin. Hazard symbols (according to GSH):

SPL Buffer contains SDS (Sodium dodecyl sulfate, >1 - <5%; CAS number 151-21-3; H-phrases: H319; P-phrases: P270 - P273 - P280 - P302 + P352 - P304 + P340 - P310. SDS can cause serious eye irritation. Hazard symbols (according to GSH):

4 Additional materials required

- DTT (per kit: ca. 500 µl of a 60 mM DTT solution)
- Gel electrophoresis system and gels
- Imaging system for detection of blue and red fluorescence

5 Introduction

Smart Protein Layers (SPL) is a technology for stain-free, quantitative and standardized analysis of protein gels. SPL allows to precisely compare data derived from different experiments.

SPL offers:

- Stain-free, highly-sensitive and standardized protein visualization on gels,
- Protein content equalizer (load by volume, inaccurate protein determination, loss of sample),
- Precise comparison of different experiments.

SPL is based on three components:

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).

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

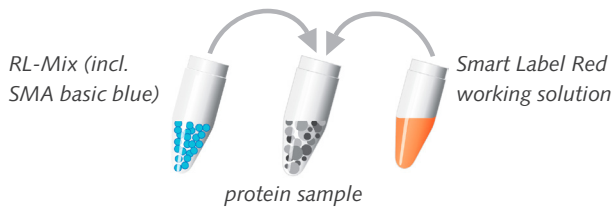
The bi-fluorescent Calibrator (CAL) is the standard for comparison between different gels or blots. CAL works also as bi-fluorescent molecular weight marker (80 kDa; 25 kDa; 12.5 kDa).

6 General protocol of the SPL analysis

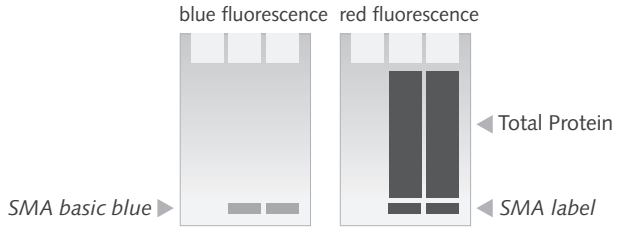
Be sure that all required materials and reagents are present before you start.

Overview Workflow SPL Analysis

1 Add reaction & labeling *(RL)-Mix (incl. SMA)* & *Smart Label* to your protein



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



6.1 Sample preparation and Smart labeling of protein samples

6.1.1 Preparation of the Smart Label working solution

- Allow vials containing Smart Label Reagent A and Smart Label Reagent B to warm up to ambient temperature. Smart Label Reagent B contains molecular sieves to avoid water pollution.
- Spin down briefly.
- To solubilize Smart Label add 15 µl of Smart Label Reagent B to Smart Label Reagent A, mix and spin down briefly.
- Transfer all liquid from Smart Label Reagent A to Smart Label Reagent B, mix and spin down briefly.

  Note: This step ensures optimum stability of Smart Label working solution for up to 6 months.

Now Smart Label Reagent B contains ready-to-use Smart Label working solution.

Store unused Smart Label working solution for up to 6 months at -20°C to -80°C.

6.1.2 Preparation of the SPL reaction & loading mix (RL-mix)

For preparation of the RL-mix, mix

6 µl of SPL buffer,
2 µl of 60 mM DTT (newly prepared) and
2 µl of SMA basic size S or L

and spin down briefly. The RL-mix is now ready-to-use.

Note: Preparation of the RL-mix as a master mix is recommended. n protein samples = n+1 x mastermix

Note: SPL buffer contains SDS, which may precipitate during freezing. Allow SPL Buffer to warm to ambient temperature and mix until SDS is redissolved completely. Precipitation does not influence SPL buffer quality.

6.1.3 Labeling of protein samples

- Transfer up to 10 µl of protein sample (containing up to 100 µg of protein) in a fresh micro centrifugation tube.

  Note: If your sample contains more than 10 µl or 100 µg of protein scale up the labeling assay.

- Add 10 µl of RL-mix (see step 1b), mix and spin down briefly.

- Add 1 µl of Smart Label working solution (reagent B; see step 7.1.1), mix and spin down briefly.

- Perform denaturation and labeling of proteins by incubation for 5 min at 95°C. The protein samples are now ready for gel electrophoresis.

  Note for compatibility of protein samples:
  Smart labeling is compatible with all commonly used buffer systems. Please avoid amines or ammonium salts with higher concentrations than 400 mM.

  Note for choosing appropriate size of SMA basic:
  SMA is available in size S (12.5 kDa) and size L (80 kDa). For gels containing <15% AA/BisAA use SMA basic size L and for gels containing >15% AA/BisAA use SMA basic size S. Nevertheless, the size of SMA basic should differ from size of target protein as well as highly abundant proteins within your protein sample.

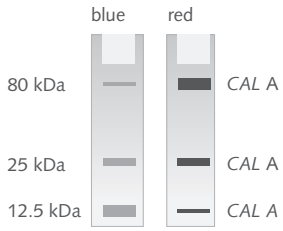
6.1.4 Usage and preparation of CAL

Use the Calibrator CAL A for experiment-to-experiment comparisons of fluorescence signals.

CAL A emits a red and blue fluorescence and is applied to every gel in equal amounts. CAL A comprises 3 polypeptides (12.5 kDa, 25 kDa and 80 kDa) in different amounts (to match various imaging settings e.g. exposure time). It can be used for experiment-to-experiment comparisons as well as as molecular weight marker.

Note: A charge depending normalization factor is available for the usage of different charges of CAL.

Calibrator A:



Preparation of CAL

Preparation of CAL as a master mix is recommended.
n x gels = n x master mix.

- Transfer 8 µl CAL per gel into a fresh micro centrifugation tube.

- Add 2 µl of 60 mM DTT (freshly prepared) per gel, mix and centrifuge briefly.

- Perform denaturation of proteins by heating for 5 min at 95°C. CAL is now ready for gel electrophoresis.

- Apply 10 µl of the prepared CAL to each gel.

6.2 Gel electrophoresis and fluorescence detection

6.2.1 Gel electrophoresis

Apply the appropriate amount of protein sample to your gel. Apply 10 µl of CAL (prepared according to 6.1.4). Perform electrophoresis as usual.

Note: When using SMA size S (= 12.5 kDa) make sure that electrophoresis has to be stopped before the SMA size S bands have migrated out of the gel.

Note: Apply the same amount of CAL to every gel.

6.2.2 Fluorescence Imaging

Imaging of fluorescence signals should be performed directly after electrophoresis without additional fixing or staining steps.

Detect fluorescence signal of total protein and SMA basic:

SMA basic: blue
Total protein (incl. SMA label): red

Optimal detection parameters (exposure time of camera or PMT of the photomultiplier): most intense signal of your samples (total protein or SMA basic or SMA label) should be marginally below saturation. Do not use CAL for optimization.

Excitation and emission parameters:

	max. excitation [nm]	max. emission [nm]
blue	496	520
red	650	665

Note: Indicate acquired imaging files according to the product guide "Data Processing and Evaluation" to enable fast and easy processing of your data.

Notes for post-electrophoretic applications: see 7.

6.3 Processing and evaluation of acquired data

See product guide "SPL Data Processing and Evaluation"

7. Post-electrophoretic applications

Fixation and storage of the gel
Fixate proteins in the gel for 30 min using 40% (v/v) ethanol and 10% (v/v) acetic acid. Store gels in 25% (v/v) ethanol and 3% (v/v) glycerol. In case of ready- to-use gels please note the manufacturer manual.

Staining
SPL labeled proteins can be stained with all visible staining like Coomassie staining or silver staining. Please note that visible staining may mask the fluorescence signals.

Mass spectrometry
The SPL fluorescence labeling has no influence on the later identification by mass spectrometry analysis. It does not affect the efficiency of enzymatic digestions and or the sequence coverage compared to unlabeled proteins.

Western Blot analysis
SPL gels can be blotted as usual immediately after gel electrophoresis. The fluorescence labeling is transferred to the blotting membrane together with the proteins and can be used directly as a transfer control by simple fluorescence imaging. The subsequent treatment of the membrane (blocking, incubation with primary and secondary antibodies, etc.) is not influenced by the SPL labeling.

8 Symbols used

According to DIN EN ISO 15223-1:

	Consult instructions for use (product guide)
	Storage conditions (temperature)
	Expiry date
	Product number
	LOT number
	Sufficient for <n> reactions
	Manufacturer
	Medical device
	Unique Device Identifier Code