NH DyeAGNOSTICS

Product Guide

Saturn-2D™ Labeling Kit XS

Titration Kit

Product no. PR30

NH DyeAGNOSTICS GmbH
Weinbergweg 23
D-06120 Halle

Technical Support
Fon: +49 (0) 345-2799 6413
e-mail: service@dyeagnostics.com
www.dyeagnostics.com

Revised 09/2017 (1)

FOR RESEARCH USE ONLY

1 Products and content

	Saturn-2D™ XS Titration Kit
Product no.	PR30
S-Dye 200	1x XS (for 3 reactions)
S-Dye Solvent	1x
TCEP	1x
ddH_2O	1x
S-Dye Low-retention Tubes & Tipps	1x

2 Storage and stability

Store S-Dyes, S-Dye Solvent, TCEP and ddH_2O at -20°C bis -80°C in the dark.

Best before: see kit package

Short-term storage (< 2h) of dissolved S-Dyes: +2 to +8°C. Long-term storage of dissolved S-Dyes: -20 to -80°C. Use dissolved S-Dyes within 3 weeks. Avoid repeated freeze-thaw-cycles. Store labeled protein at -20°C to -80°C. Labeled protein can be stored at least for 3 month.

Short-term storage (< 2h) of dissolved S-Dyes: +2 to +8°C. Long-term storage of dissolved S-Dyes: -20 to -80°C. Use dissolved S-Dyes within 3 weeks. Avoid repeated freeze-thaw-cycles. Store labeled protein at -20°C to -80°C. Labeled protein can be stored at least for 3 month.

3 Safety instructions

The S-Dye Solvent contains dimethylformamide (DMF, $HCON(CH_3)_2$, CAS No: 68-12-2) and is harmful by inhalation, ingestion or skin contact.

4 Additional materials required:

- Saturn-2D™ compatible sample buffer (see 7.1)
- per sample: ca. 0,5 mg DTT (Dithiothreitol)
- 2D gel electrophoresis system (incl. required material)
- opt.: Low-fluorescent glass cassette or VELUM GOLD Precast 2D Gels (PR237, PR241)
- Imaging system for detection of green and red
- opt.: Software for data evaluation (e.g. Delta 2D; available at www.dyeagnostics.com)

5 General Information

The S-Dye200 and S-Dye300 are maleimide-activated high performance fluorescent dyes for labeling of proteins. After reduction of the thiol-groups of the cysteines, the S-Dyes are covalently bound to the proteins. The proteins than can be separated by one or two dimensional gel electrophoresis or by liquid chromatography and specifically recognized by their characteristic fluorescent label.

Since each protein sample may differ in its content of cysteines, the determination of adequate amounts of TCEP and S-Dye is required. Use the Saturn-2D™ Titration Kit for the evaluation of the optimal labeling parameters for subsequent Saturn-2D™ analyses.

20 µg of protein from your sample along with four 2D-SDS gels (titration gels; and optional one 1D-SDS gel) are required.

6 Overview: Determination of optimal labeling parameters

1. Solubilisation of proteins in Saturn-2D™ compatible sample buffer
2. Preparation of the TCEP reducing solution
3. Preparation of the S-Dye Working Solution
4. Labeling of protein samples for titration gels
5. Fluoreszenz-Imaging
6. Comparison of spot pattern of the titration gels.

For questions contact us at service@dyeagnostics.com.

7 Detailled protocol for determination of optimal labeling parameters

7.1 Solubilisation of proteins in Saturn-2D™ compatible sample buffer

For optimum labeling results, make sure that the protein is dissolved in a Saturn-2D™ compatible sample buffer (10-100 mM Tris, or HEPES at pH < 8 (optimum: pH 7.5)). Avoid buffers containing primary amines or thiols. Make sure that the protein concentration of the samples is in the range of 0.55 - 10 µg/µl.

Note: If the protein concentration is below 0.55 µg/µl precipitate the sample and dissolve it in a lower volume, or in the case of higher concentrations, dilute the sample with your S-Dye compatible sample buffer.

7.2 Preparation of the TCEP reducing solution

- Pipette 400 µl of the sterile ddH_2O into the vial containing TCEP.
- Vortex and spin down briefly.
- The TCEP reducing solution is now ready for further use.

Note: We recommend the usage of the provided TCEP instead of DTT for the reduction of proteins. DTT interferes with the S-Dyes and has to be removed for the subsequent labeling reaction (e.g. by dialysis).

7.3 Preparation of the S-Dye Working Solution

Note: Dissolve S-Dyes immediately before use. Store dissolved S-Dyes for short-term (< 2 h) at +2 to +8°C. Long-term storge: -20°C to -80°C. Use dissolved S-Dyes within 3 weeks. Avoid repeated freeze-thaw-cycles.

Note: We recommend the usage of the provided S-Dye low retention pipette tips and micro centrifuge tubes.

- Allow vial to warm up to ambient temperature (approx. 5 minutes).
- Spin down vials briefly.
- Dissolve S-Dye:

  S-Dye 200 for 3 reactions (XS):

  in 12 µl S-Dye Solvent per XS vial (PR30).
- Mix (vortex) and spin down briefly.
- The S-Dye working solution is now ready for further use.

7.4 Labeling of protein samples for titration gels

Note: All experimental steps including protein samples should be performed on ice.

Label the protein samples or a mixture of protein samples, that has to be analyzed using Saturn-2D™ technology.

To determine the optimal TCEP and S-Dye amounts for respective sample type please refer also table 1 below and as follows:

- Adapt the protein concentration of each sample to 0.55 µg/µl with a Saturn-2D™ compatible sample buffer.
- Add to 9 µl (corresponding to 5 µg of protein) of your protein solution the respective volume of TCEP.
- Vortex and spin down briefly.
- Incubate for 1 h at 35˚C.
- Vortex and spin down briefly.
- Add the respective volume of S-Dye working solution to the reduced sample
- Vortex and spin down briefly.
- Incubate for 1 h at 35˚C.
- Vortex and spin down briefly.
- Quench the labeling reaction by adding DTT to a final concentration of 65 mM (e.g. by adding the equal volume of an IEF loading buffer containing 130 mM DTT).
- The sample can be loaded directly on an IPG strip subsequent to addition of the corresponding volume of rehydration buffer.
- optional: check for labeling bei 1D SDS-PAGE (recommended amount of protein: 0,1 µg per lane).

7.5 Fluoreszenz-Imaging

Imaging parameters (e.g. voltage of the photomultiplier tube (PMT) or exposure time of the CCD camera) are dependent on the fluorophore, the gel quality and constitution of the sample. For best fluorescence performance optimize detection parameters for each dye by imaging the gel with a low resolution scan. Signal intensity of the most abundant spot(s) should be marginally below saturation (saturation: 65,535 grey values for 16 bit).

Acquire the fluorescent image of the S-Dye labeled protein gels after finishing SDS-PAGE.

Please find further information on www.dyeagnostics.com/

S-Dye excitation and emission parameters

S-Dye	max. excitation [nm]	max. emission [nm]
S-Dye200	555	576

7.6 Comparison of spot pattern of the titration gels.

Compare the spot pattern of all images of your titration experiment. The S-dyes bind covalently to proteins and will cause a mass and charge shift of labeled protein in comparison to unlabeled protein. Unefficient labeling (amount of S-Dye and TCEP is too low) will result in horizontal or diagonal streaking (for images please visit www.dyeagnostics.com/site/en/products/saturn-2d/ - product guide Saturn-2D™ XS Titration Kit).

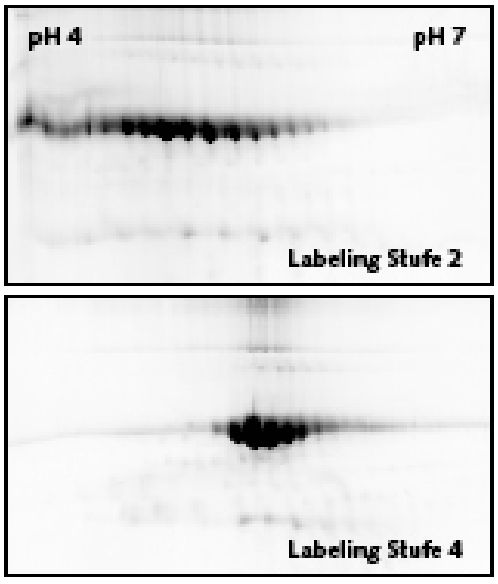

Figure 1: Titration experiment of BSA. Unefficient labeling (here degree of labeling 2) will result in dramatically shifted spot pattern. In case of BSA optimal labeling will occur using degree of labeling 4.

8 Post-electrophoretic applications

Gels stored within low fluorescent glass cassettes (product no PR03 and PR04) can be imaged up to 24 h after finishing SDS-PAGE. Otherwise, fixate the gel for 30 min in fixing solution (40% ethanol/ 10% acetic acid) and than store the gel in a solution containing 25% ethanol/ 3% glycerol in the dark (incubate for 15 min in water before scanning). For pre-cast gels see manufacturers' recommendations.

S-Dye label does not interfere with protein identification by mass spectrometry, enymatic digestions or sequence coverage.

S-Dye labeled proteins can be blottet and stained with common stains (note: observe detection limits as well as excition and emission parameters of the stains; post-electrophoretic stains may mask S-Dye fluorescence signals).

Table 1: Determination of optimal labling parameters

Degree of labeling	add protein		add TCEP		add S-Dye 200		add stop buffer*	No. of 2D-Gel
1	5 µg	Add Saturn-2D™ compatible sample buffer (see 7.1) zu a final volume of 9 µl.	0.5 µl	Vortex and spin down briefly. Incubate for 1 h at 35°C.	1.0 µl	Vortex and spin down briefly. Incubate for 1 h at 35°C.	10.5 µl	1
2	5 µg		1.0 µl		2.0 µl		12.0 µl	2
3	5 µg		1.5 µl		3.0 µl		13.5 µl	3
4	5 µg		2.0 µl		4.0 µl		15 µl	4

* see 7.4