NH DyeAGNOSTICS

Product Guide

Saturn-2D™ Labeling Kit

Product no. PR31, PR32, PR33

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1 Products and content

	Saturn-2D™ Kit 4S	Saturn-2D™ Kit 8S	Saturn-2D™ Kit 8S Prep
Product No.	PR31	PR32	PR33
S-Dye 200	1x 4S	2x 4S	2x 4S + 1x 40S
S-Dye 300	1x 4S	2x 4S	2x 4S
S-Dye Solvent	1x	1x	2x
TCEP	1x	1x	2x
ddH_2O	1x	1x	2x
S-Dye Low-retention Tubes & Tipps	1x	1x	2x

The declaration 4S and 8S indicates the number of large 2D gels that can be performed with the kit.

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.

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 7.2)
- 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
- 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.
For 2D gel analyses a minimum of four 2D gels (4S kit) respectively eight 2D gels (8S kit) with 5 µg of S-Dye200 and S-Dye300 labeled proteins can be performed.

6 Overview: Saturn-2D™ Labeling

1. Experimental design

2. Solubilisation of proteins in Saturn-2D™ compatible sample buffer
3. Preparation of an internal standard (IS)
4. Preparation of the TCEP reducing solution
5. Preparation of the S-Dye Working Solution
6. Determination of optimal labeling parameters
7. Labeling of protein samples for Saturn-2D™ analysis
8. Fluoreszenz-Imaging

For questions contact us at service@dyeagnostics.com.

7 Detailled protocol for Saturn-2D™ Labeling

7.1 Experimental design

For comparison of two protein samples (e.g. wild type vs. mutant) use two 2D gels (plus replicates). Analyse one sample and the internal standard (IS) within one 2D gel. The IS consists of a mixture of all protein samples of your experiment. This allows easy gel-to-gel-comparison. A dye swap is not required for Saturn-2D™ analysis.

Example: Comparison of sample A and sample B

Gel 1:

sample A (5 µg) labeled with S-Dye300 +
IS (2.5 µg sample A + 2.5 µg sample B) labeled with S-Dye200

Gel 2:

sample B (5 µg) labeled with S-Dye300 +
IS (2.5 µg sample A + 2.5 µg sample B) labeled with S-Dye200

7.2 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.

7.3 Preparation of the Iternal Standard (IS)

The internal standard (IS) represents a mixture of all protein samples of your experiment and allows easy gel-to-gel comparison. We re-commend to use S-Dye200 as fluorescent label for the IS.

For n (n = number of required 2D gels) 2D gels you produce a pool of equal protein amounts representing all samples of your experiment. Set the protein concentration to 0,55 µg/µl using a Saturn-2D™ compatible sample buffer. Label this mixture using S-Dye200.

Example:

n = 1 gel, protein sample A and B

Mix 2,5 µg of protein from sample A with 2,5 µg of protein from sample B, adjust protein concentration to 0,55 µg/µl and label with S-Dye 100 (according to pretested optimal labeling parameters (7.6).

n= 5 gels, protein sample A and B

Mix 12,5 µg of protein from sample A with 12,5 µg of protein from sample B, adjust protein concentration to 0,55 µg/µl and label with S-Dye 200 (according to pretested optimal labeling parameters (7.6). Distribute the labeled internal standard in equal amounts of 5 µg to the five 2D gels or IPG strips.

7.4 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).

7.5 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 vials to warm up to ambient temperature (approx. 5 minutes).
- Spin down vials briefly.
- Dissolve S-Dyes:

S-Dye 200 / 300 for 4 reactions (4S):

in 16 µl S-Dye Solvent per 4S vial (PR31, PR32, PR33).

S-Dye 200 Prep for 40 reactions (40S):

in 160 µl S-Dye Solvent per 40S vial (PR33). According to pretested labeling parameters (7.6) 200 - 800 µg of protein can be labeled.

- Mix (vortex) and spin down briefly.
- The S-Dye working solution is now ready for further use.

7.6 Determination of optimal labeling parameters

Since each protein sample may differ in its content of cysteines, the determination of adequate amounts of TCEP and S-Dye is required. We recommend a pretest for which 20 µg of protein from your sample along with four 2D-SDS gels (and optional one 1D-SDS gel) are required.

For this pretest adapt the protein concentration of your sample to 0.55 µg/µl using a S-Dye compatible buffer, and subdivide the sample according to table 1 (see page 3).

Compare the spot pattern of all images of your pretest. The S-dyes bind covalently to proteins and will cause a mass and charge shift of labeled protein in comparison with 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).

NEW
For pretesting use new Satrun-2D™ XS Titration Kit (product no. PR30).

7.7 Labeling of protein samples for Saturn-2D™ analysis

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

Label the protein samples after evaluating the optimal TCEP and S-Dye amounts for respective sample type. Please refer also to table 2 on page 4.

- 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 TCEP volume according to pretested labeling parameters (7.6).
- Vortex and spin down briefly.
- Incubate for 1 h at 35˚C.

Note: For labeling assays containing more than 5 µg protein scale up the reaction maintaining the ratio of protein/ TCEP/ S-Dye.

- Vortex and spin down briefly.
- Add S-Dye working solution according to the pretestet labeling parameters
- 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 appropriate volume of rehydration buffer.
- optional: check for labeling bei 1D SDS-PAGE (recommended amount of protein: 0,1 µg per lane).

7.8 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
S-Dye300	649	664

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 labeling 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 7.2) 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 7.7

Table 2: Overview Saturn-2D™ labeling of protein samples

Degree of labeling	add protein		add TCEP		add S-Dye 200	add S-Dye 300		add stop buffer*
1	5 µg	Add Saturn-2D™ compatible sample buffer (see 7.2) 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
	5 µg		0.5 µl		---	1.0 µl		10.5 µl
2	5 µg		1.0 µl		2.0 µl	---		12.0 µl
	5 µg		1.0 µl		---	2.0 µl		12.0 µl
3	5 µg		1.5 µl		3.0 µl	---		13.5 µl
	5 µg		1.5 µl		---	3.0 µl		13.5 µl
4	5 µg		2.0 µl		4 µl	---		15.0 µl
	5 µg		2.0 µl		---	4 µl		15.0 µl

* see 7.7