



# Product Guide

## Saturn-2D™ Labeling Kit

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Rev. 01/2012

### Saturn-2D™ Labeling Kit

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 then can be separated by one or two dimensional gel electrophoresis or by liquid chromatography and specifically recognized by their characteristic fluorescent label.

For 2D 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.

For further information, recommendations and useful tips, profit from our free remote NHD Expert Coaching at [service@dyeagnostics.com](mailto:service@dyeagnostics.com).

Please avoid unnecessary exposure of the S-Dyes to excess of light.

### Kit content

- 1 vial containing S-Dye200
- 1 vial containing S-Dye300
- 1 vial containing S-Dye solvent
- 1 vial containing TECP (Tris-(2-carboxyethyl)phosphine-hydrochloride)
- 1 vial containing ddH<sub>2</sub>O, sterilized
- S-Dye low retention pipette tips (Mass Spec compatible)
- S-Dye low micro centrifuge tubes (Mass Spec compatible)

Note: Each sample requires approx. 0.5 mg of DTT (Dithiothreitol).

### Caution

The S-Dye solvent contains dimethylformamide (DMF, HCON(CH<sub>3</sub>)<sub>2</sub>, CAS No: 68-12-2) and is harmful by inhalation, ingestion or skin contact.

### Protocol for protein labeling

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

### S-Dye compatible buffers and required protein concentration

For optimum labeling results, make sure that your protein is dissolved in a Saturn-2D™ compatible lysis 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 your samples is between 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 lysis buffer.

### Preparation of the TCEP reducing solution

1. Pipette 400 µl of the sterile ddH<sub>2</sub>O into the vial containing TCEP.
2. Vortex and centrifuge briefly.
3. Your TCEP reducing solution is now ready for further use.

### Note

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

### Preparation of the S-Dye working solution

1. Allow vials to warm up to ambient temperature in the dark (approx. 5 minutes).
2. Spin down vials briefly.
3. Dissolve S-Dyes in
  - 16 µl of S-Dye solvent for the 4S kit.
  - 32 µl of S-Dye solvent for the 8S kit.
4. Mix (vortex) and centrifuge briefly. The S-Dye working solution is now ready for further use.

### Note

After centrifugation of the tubes a minor portion of S-Dye may remain on edge of the conical part of the tube.

To dissolve this moiety, put the S-Dye solvent on the edge of the ring (figure 1). Guide the drop along the ring with the pipet tip (360°) and then draw back the drop with the dissolved S-Dye into the pipet tip. Dispose the S-Dye solvent onto the tube wall of the conical part of the tube (figure 2).



Fig.1



Fig.2

## Pretest for the evaluation of optimal labeling parameters

Since each protein sample may differ in its content of cysteines, the determination of the adequate amounts of TCEP and S-Dye is required.

We recommend a pretest for which you require 40 µg protein from your sample along with four 2D-SDS gels (and optional one 1D-SDS gel).

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

If TCEP and S-Dye amounts are optimal, all corresponding protein spots are matched in the merged gel images. Images overlay requires suitable software (see also [www.dyeagnostics.com/site/technology/software](http://www.dyeagnostics.com/site/technology/software)).

## S-Dye excitation and imaging properties

| S-Dye    | excitation max. [nm] | emission max. [nm] |
|----------|----------------------|--------------------|
| S-Dye200 | 555                  | 576                |
| S-Dye300 | 649                  | 664                |

### Note

Imaging parameters (e.g. voltage of the photomultiplier tube (PMT) or exposure time of the CCD camera) are dependent on gel quality and constitution of the sample. For best fluorescence performance of the S-Dyes 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.

Table 1.

| # reaction (vial) | add protein* |          | add TCEP-solution |            | add S-Dye200 | add S-Dye300 |            | add stop buffer**  |          | required vol. for 0.1 µg | 2D gel no. |
|-------------------|--------------|----------|-------------------|------------|--------------|--------------|------------|--------------------|----------|--------------------------|------------|
| 1                 | 5 µg         | Fill up  | 0.5 µl            | Vortex     | 1.0 µl       | -            | Vortex     | 10.5 µl (optional) |          | 0.42 µl                  | 1          |
| 2                 | 5 µg         | with     | 0.5 µl            | and spin   | -            | 1.0 µl       | and spin   | 10.5 µl            | Check    | 0.42 µl                  | 1          |
| 3                 | 5 µg         | sample   | 1.0 µl            | down       | 2.0 µl       | -            | down       | 12.0 µl            | the      | 0.48 µl                  | 2          |
| 4                 | 5 µg         | buffer   | 1.0 µl            | briefly.   | -            | 2.0 µl       | briefly.   | 12.0 µl            | labeling | 0.48 µl                  | 2          |
| 5                 | 5 µg         | to a     | 1.5 µl            | Incubate   | 3.0 µl       | -            | Incubate   | 13.5 µl            | with a   | 0.54 µl                  | 3          |
| 6                 | 5 µg         | volume   | 1.5 µl            | for 1 h at | -            | 3.0 µl       | for 1 h at | 13.5 µl            | 1D       | 0.54 µl                  | 3          |
| 7                 | 5 µg         | of 9 µl. | 2.0 µl            | 37°C.      | 4.0 µl       | -            | 37°C in    | 15.0 µl            | gel***.  | 0.60 µl                  | 4          |
| 8                 | 5 µg         |          | 2.0 µl            |            | -            | 4.0 µl       | the dark.  | 15.0 µl            |          | 0.60 µl                  | 4          |

\* Protein from the same type of sample or protein of a pooled mix of samples to be analyzed.

\*\* In order to quench the labeling reaction, use a 2D rehydration buffer containing 130 mM DTT and 2% ampholyte.

\*\*\* Recommended protein amount: 0.1 µg per lane.

## Labeling of protein samples

Label your protein samples after evaluating the optimum TCEP and S-Dye amounts for respective sample type.

1. Adapt the protein concentration of your sample to 0.55 µg/µl with your S-Dye compatible lysis buffer.
2. Add to 9 µl (corresponding to 5 µg of protein) of your protein solution the optimum TCEP volume you evaluated during the pretest. Incubate for 1 h at 37°C after briefly vortexing and subsequent briefly centrifuging.
3. Collect the reduced sample by brief centrifugation. Add your evaluated optimum amount of S-Dye working solution to the reduced sample, vortex, and spin down briefly. Incubate for 1 h at 37°C in the dark.
4. Centrifuge the labeling mixture 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 after addition of the corresponding volume of rehydration buffer.

## Storage

Store S-Dyes at -20°C to -80°C in the dark. Label proteins within four weeks after solubilization of S-Dyes.

Store labeled proteins at -80°C.

Best before: see packaging

### Disclaimer

Saturn-2D™ is a novel technique for multiplex-fluorescence 2D gel electrophoresis. However, the Saturn-2D™ technique and the corresponding S-Dyes (Saturn-2D™ Labeling Kit) are not suitable for Ettan® DIGE analysis offered by GE Healthcare, since the Ettan® DIGE analysis requires that the dyes used therein are matched with respect to their electrophoretic mobility which does not apply to the technique offered by NH DyeAGNOSTICS.