NH DyeAGNOSTICS

Product Guide

Saturn-2D™ REDOX Labeling Kit 6R

Product no. PR412

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1 Products and contents

Pack 1		Pack 2	
S-Dye 200	2x 3R	Redox Matrix (ready to use, 50%)	1x
S-Dye 300	2x 3R	Redox Wash Buffer	1x
S-Dye Solvent	1x	Redox Labeling Buffer	1x
TCEP	1x		
ddH_2O	1x		
Redox Stop Solution	1x		
Cysteine interacting compound (CinC)	2x 3R		
CinC solvent	1x		
additionally			
S-Dye Low-retention Tubes & Tipps		1x	

2 Storage and stability

Store S-Dyes, S-Dye Solvent, CinC, CinC Solvent TCEP, Redox Stop Solution and ddH_2O (Pack 1) at -20°C bis -80°C in the dark.

Store Redox Matrix, Redox Wash Buffer und Redox Labeling Buffer (Pack 2) at +2 bis +8°C.

Best before: see kit package

Short-term storage (< 2h) of dissolved S-Dyes and CinC: +2 to +8°C. Long-term storage of dissolved S-Dyes and CinC: -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 and CinC Solvent contains dimethylformamide (DMF, $HCON(CH_3)_2$, CAS No: 68-12-2) and is harmful by inhalation, ingestion or skin contact.

The Redox Matrix contains preservative agents (corrosive).

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)
- Centrifuge (suitable for 2 ml vials; at least 800 xg)

5 General Information

Saturn-2D™ REDOX is a novel technology for the simple visualization of complex stress response of the cellular proteome. Samples differing in their REDOX potential are specifically labeled and then compared.

The existing problem of inconsistent spot matching - unequal binding of fluorescent dyes to proteins differing in their REDOX-potential - is solved by a special Cys-interacting compound (CinC).

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 these residues. The proteins then can be separated by one or two dimensional gel electrophoresis and specifically recognized by their characteristic fluorescent label. Six 2D gels can be performed using Saturn-2D™ Redox Kit 6R (labeling of 6 x 5 µg of protein (6R kit) with S-Dye200 and S-Dye300, respesctively).

6 Overview: Saturn-2D™ REDOX Analysis

1. Experimental design
2. Solubilisation of proteins in a Saturn-2D™ REDOX compatible sample buffer
3. Preparation of the TCEP reducing solution
4. Preparation of the S-Dye working solution
5. Preparation of the CinC working solution
6. Redox labeling
7. Removal of excessive CinC
8. Preparation of the internal standard (IS)
9. Fluorescence imaging

For questions contact us at service@dyeagnostics.com.

7 Detailed protocol for Saturn-2D™ REDOX Analysen

7.1 Experimental Design

For comparison of two samples (e.g. control vs. stressed) two 2D gels (plus replicates) are required. Every samples is labeled with the CinC, reduced and finally labeled with the S-Dye (see scheme 1). The CinC is blocking all recently reduced cysteines and the S-Dye shows all recently oxidized cysteines (except for: sulfinic and slfonic acid). One sample and one internal standard (IS) is analyzed per 2D gel. The IS represents a mixture of all samples of the experiment (totally reduced) and allows easy gel-to-gel comparison. A dye-swap is not neccessary. However, differences in protein expression may mask the redox state of your samples. We therefore recommend to perform a subsequent Saturn-2D™ analysis to ensure your results.

7.2 Solubilisation of proteins in a Saturn-2D™ REDOX compatible sample buffer

For optimal labeling results, make sure that the protein is dissolved in a Saturn-2D™ REDOX 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 at least 1 µg/µl.

Note: If the protein concentration is below 1 µg/µl, precipitate the sample and dissolve it in a lower volume S-Dye compatible sample buffer.

7.3 Preparation of the TCEP reducing solution

- Pipette 400 µl of the sterile ddH_2O into the vial containing TCEP.
- Vortex and centrifuge 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).

7.4 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 in

  16 µl of S-Dye solvent per vial 3R.
- Mix (vortex) and centrifuge briefly. The S-Dye working solution is now ready for further use.

7.5 Preparation of the CinC working solution

Note: Dissolve CinC immediately before use. Store dissolved CinC 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 CinC in

  16 µl of CinC solvent per 3R vial.

- Mix (vortex) and centrifuge briefly. The CinC working solution is now ready for further use.

7.6 Redox Labeling (see also Tabelle 1)

1. Adapt the protein concentration of each sample to 0.55 µg/µl with Redox labeling buffer.
2. Add 3 µl CinC working solution to 9 µl (corresponding to 5 µg of protein) of your protein solution. Vortex and spin down briefly, incubate for 1 h at 35°C.
3. Spin down briefly.
4. Quench the reaction by adding of 3 µl Redox stop solution. Vortex and spin down briefly, incubate for 10 min at 35°C.
5. Spin down briefly.
6. Remove excessive CinC (see Chapter 7.7).
7. Add 2.5 µl of TCEP reducing solution to your sample. Vortex and spin down briefly, incubate for 1 h at 35 °C.
8. Spin down briefly. Add 5 µl of S-Dye 300 working solution. Vortex and spin down briefly, incubate for 1 h at 35°C.
9. Spin down briefly.
10. Quench the reaction by adding of 6 µl of Redox stop solution.
11. Vortex and spin down briefly, incubate for 10 min at 35°C. Spin down briefly.
12. The sample can be loaded directly on an IPG strip after addition of the corresponding volume of rehydration buffer.

7.7. Removal of excessive CinC

Note: Redox matrix contains preservative agent (corrosive).

7.7.1 Preparation of the column:

- Mix chromatography matrix thoroughly to get a homogenous suspension.
- Load the empty spin column. Apply appropriate volume of Redox matrix (see Table below). If neccessary use more than one column and split your reaction mixture.
- Place the loaded column into a 2 ml micro centrifuge tube and-centrifuge for 2 min at 800 x g.
- Discard flow throw.

7.7.2 Equilibration of the column:

- Apply 500 µl of Wash buffer.
- Centrifuge for 1 min at 800 x g.
- Discard flow throw.
- Repeat steps 1-3 two times. Last centrifugation step: 2 min at 800 x g.

7.7.3 Sample load:

- Place equilibrated column on a new 2 ml micro centrifuge tube.
- Apply your sample carefully onto the matrix.

7.7.4Eluation of the sample:

- Centrifuge for 2 min at 800 x g.
- If neccessary combine the flow through of splitted reaction mixtures.
- Determine the volume of the flow throw (eg. by using a pipette).

reactions	**1R**	**2R**	**3R**	**4R**	**5R**	**6R**
sample volume (µl)	15	30	45	60	75	90*
matrix volume per column (µl)	288	288	432	576	720	432
no. of columns	1	1	1	1	1	2
Ansatz	**1R**	**2R**	**3R**	**4R**	**5R**	**6R**
volume of internal standard	15	30	45	60	75	90*
matrix volume per column (µl)	288	288	432	576	720	432
no. of columns	1	1	1	1	1	2

* Split reaction mixture into 2 parts

Tab. 2: Amount of Redox Matrix

7.8 Preparation of the internal standard (IS)

The IS represents a mixture of all samples of the experiment (totally reduced). The IS should be prepared in parallel to your samples (incl. CinC removal). 5 µg of protein mixture are needed per 2D gel.

7.8.1 Preparation of the IS:

- Adapt the protein concentration of each sample to 0.55 µg/µl with Redox labeling buffer.
- Make a mixture of all samples of your experiment by mixing equal amounts of protein of every sample. You need 5 µg of protein mixture per 2D gel.

7.8.2 Labeling of the IS:

The labeling of the IS sufficient for one 2D gel is described below. For labeling of IS sufficient for more than one 2D gel, please scale up your labeling reaction.

- Add 3 µl of CinC solvent to 9 µl of IS. Vortex and spin down briefly, incubate for 1 h at 35°C.
- Spin down briefly.
- Add 3 µl of sterile ddH2O. Vortex and spin down briefly, incubate for 10 min at 35°C.
- Spin down briefly.
- Perform chromatography as described in chapter 7.7.
- Add 2.5 µl of TCEP reducing solution. Vortex and spin down briefly, incubate for 1 h at 35°C.
- Spin down briefly.
- Add 5 µl S-Dye200 working solution to the reduced sample. Vortex, and spin down briefly, incubate for 1 h at 35°C.
- Spin down briefly.
- Quench the labeling reaction by adding 6 µl Redox stop solution.
- Vortex and spin down briefly, incubate for 10 min at 35°C. The sample can be loaded directly on an IPG strip after addition of the corresponding volume of rehydration buffer.

7.9 Fluorescence 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.

For MS identification of protein spots preparation of a preparative ge is recommended (Saturn-2D™ Labeling Kit 8S Prep; PR33). For processing of MS data dye modification of cysteine residues has to be taken into account (delta mass on request).

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).

		Labeling I						CinC-Removal		
reaction	sample volume (µl)	CinC (µl)	CinC Solvent (µl)		Redox Stop Solution (µl)	ddH_2O		total volume (µl)	Matrix volume (µl)	total volume (µl)
sample	9	3	---	1h at 35°C.	3	---	10 min at 35°C.	15	288	15
IS	9	---	3		---	3		15	288	15

cont.	Reduktion		Labeling II					
reaction	TCEP Reducing Solution (µl)		S-Dye 200 (µl)	S-Dye 300 (µl)		Redox Stop Solution (µl)		total volume (µl)
sample	2,5	1h at 35°C.	---	5	1h at 35°C.	6	10 min at 35°C.	28,5
IS	2,5		5	---		6		28,5

Abb. 1: Experimentelles Design für Saturn-2D™ REDOX Analysen

Tab. 1: Saturn-2D™ REDOX reaction mixture

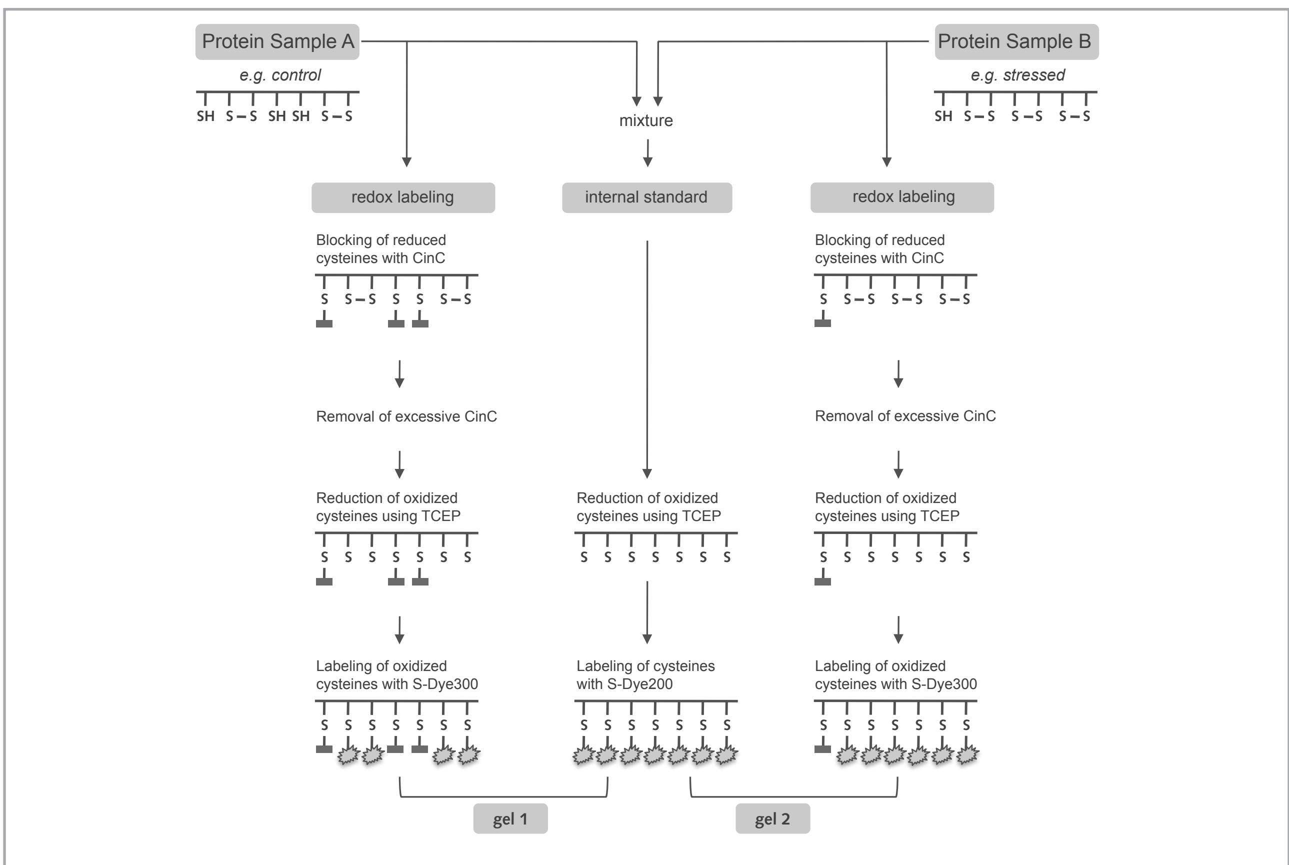

Abb. 1: Experimental design of Saturn-2D™ REDOX Analysis