

Plus appendix:

Related equipment and
Kits for precise Western blots

HUNTING FOR CANDIDATE PROTEINS

Protein labeling kits for modern 2D gel based top down proteomics

Modern gel based proteomics:

*“Combining high protein separation capacity
and the information of intact proteins
with high sensitivity labeling.”*

Dr. Christian Scharf, University of Greifswald

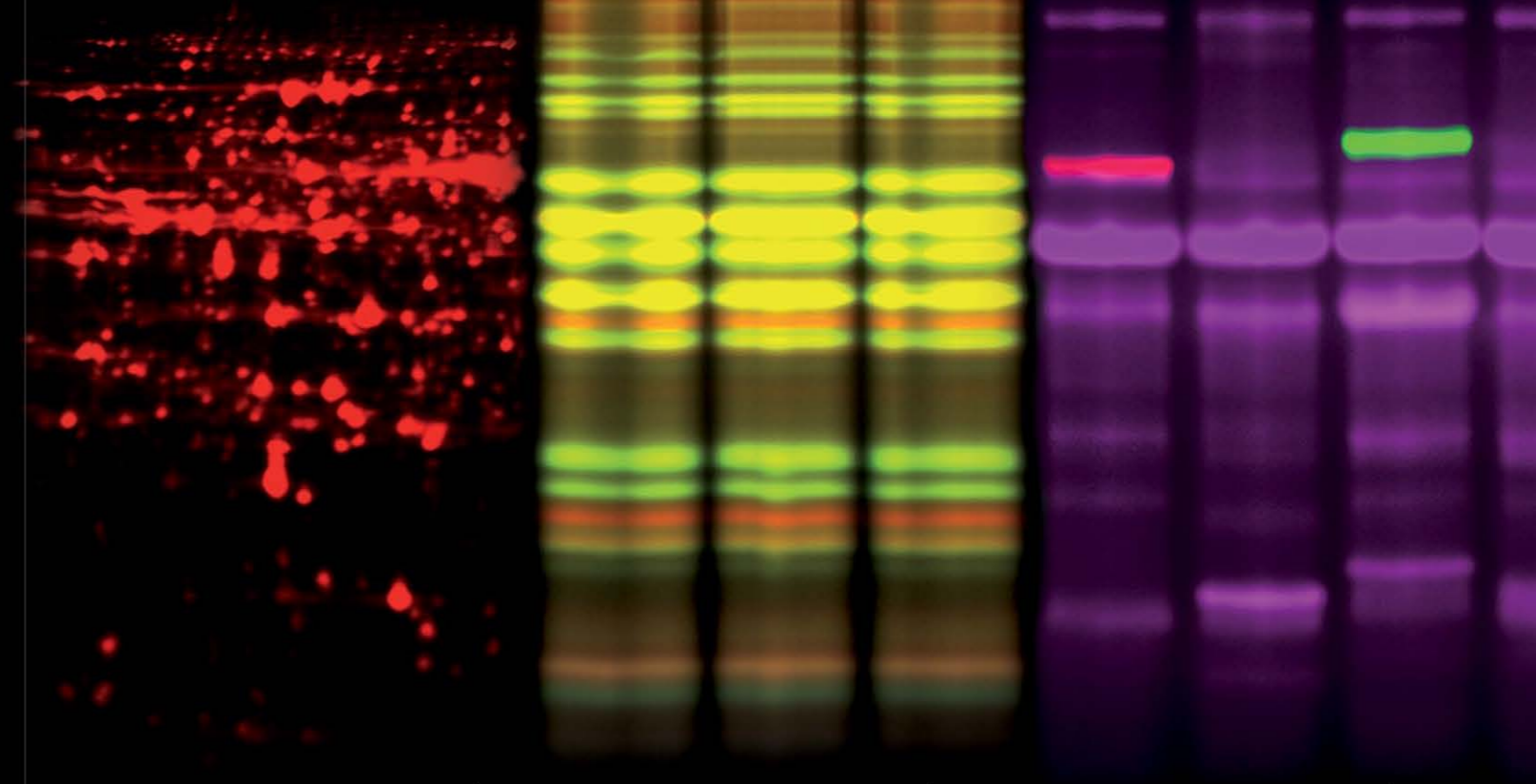


During the past 5 years NH DyeAGNOSTICS has become the world leader in fluorescence gel based top down proteomic technologies. Our products help to identify important protein biomarkers, for instance in cancer research. They also help making routine protein diagnostics of human and animal samples faster and much more reliable. Committing ourselves to develop further novel tools and to continuously advance scientific research and routine diagnostics we are eager to continue the close cooperation with our customers.

Dr. Jan Heise
CEO NH Dyeagnostics

Fluorescence labeling of proteins

T-Dyes Series



IDEAL REPLACEMENT
OF POST STAINS

COMPARE DIFFERENT
SAMPLES IN ONE WELL

NORMALIZE PROTEIN
EXPRESSION ON
WESTERN BLOTS

Instant protein visualization on gels and blots ✓

High sensitivity imaging within seconds ✓

Precise Western blot evaluation ✓

T-Green | T-Red | T-Rex | T-iRed | T-West

"Protein visualization demands dyes with high sensitivity and a high dynamic range to meet the requirements of molecular research in terms of easy handling, photostability and quality assurance."

Dr. Philippe Chafey, Institut Cochin, Paris, France

Fluorescent T-Dyes for protein labeling

Sensitive protein visualization on gels and Western blots

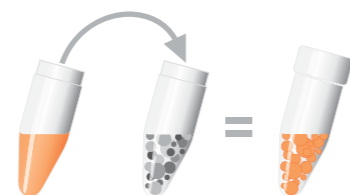
The T-Dye labels are the ideal replacement for post-stains: highly sensitive protein visualization in gels and on corresponding Western blots with best reproducibility and no staining and destaining required.

T-Dye labels are simply attached to the protein by an easy 5 min hands-on step prior electrophoresis. The T-Dyes do not interfere with antibody-protein interaction or mass spectrometry.

T-Dye protein labeling

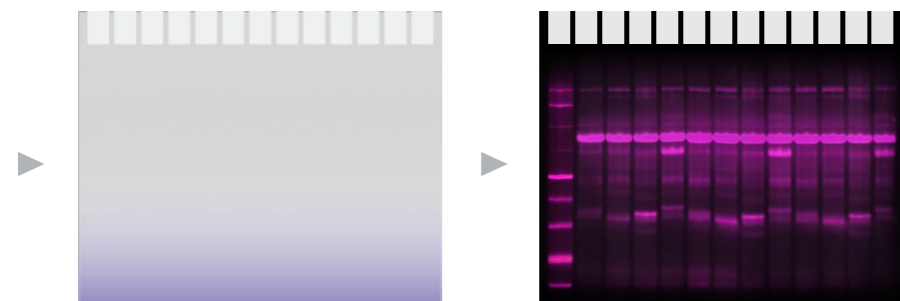
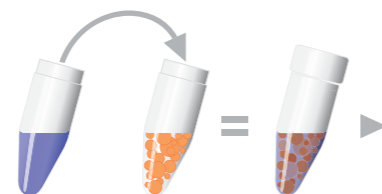
Workflow

- Add T-Dye reaction buffer and T-Dye to your sample
 - Incubate for 25 min on ice



Hands-on time 5 min
Total time 30 min

- Add T-Dye loading buffer, heat sample (4 min, 95°C)
 - Run electrophoresis
 - Image gel

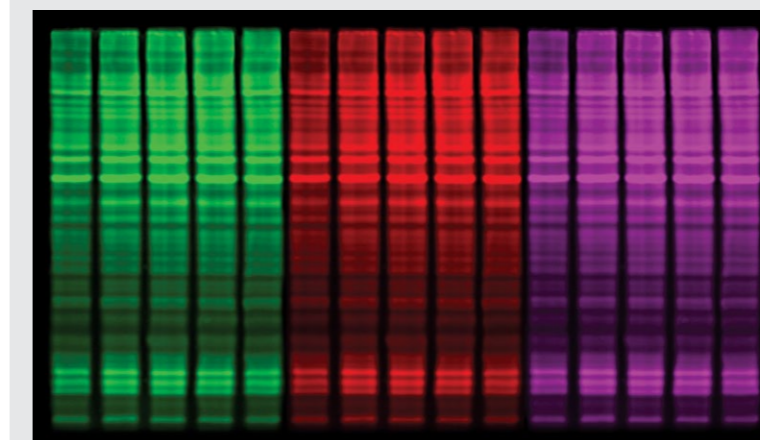


Sensitivity: 10 - 0.1 ng
Dynamic range: $10^4 - 10^5$
Exposure time: 1-2 sec

Fluorescent T-Dye protein label

Sensitive & quantifiable protein visualization

The T-Dye labeling allows you to analyze as little as 1-2 μg of T-Dye labeled protein sample per lane that can be accurately detected and quantified. Specific fluorescent labels allow the the combination of different samples within one analysis (sample multiplexing).



T-Green label T-Red label T-iRed label

1D SDS-PAGE of T-Green, T-Red and T-iRed labeled protein sample (2 μg per lane). Detection by using OctoPlus QPLEX Fluorescence Imager. Exposure times: 2 - 4.5 sec.

Characteristics

	T-Green	T-Red	T-iRed
Detection sensitivity	0.15 ng *	0.15 ng *	0.05 ng *
Dynamic range	$10^4 - 10^5$	$10^4 - 10^5$	$10^4 - 10^5$
Photostability	+++	+++	+++
Suitable for WB **	+++	+++	+++
Excitation max.	559 nm	650 nm	740 nm
Emission max.	585 nm	665 nm	760 nm
Filter settings	G-Dye200, Cy3, Alexa 555	G-Dye300, Cy5, Alexa 647	G-Dye400, LiCOR 700
Kit content	T-Green Rg. A+B Reaction Buffer Loading Buffer	T-Red Rg. A+B Reaction Buffer Loading Buffer	T-iRed Rg. A+B Reaction Buffer Loading Buffer

+++ Superior performance, * Sensitivity depending on imaging system, ** Western blot

T-West™ Fluorescence-based precise Western blots



For Western blot analysis visualization of the protein transferred onto the blot can be achieved by using various stains. However stains may have disadvantages e.g. additional labor for staining/ destaining, background problems, reproducibility, dynamic range.

T-West™ provides a powerful alternative based on fluorescent protein labels (T-Dyes). It allows for proteins to be easily visualized and quantified in gels and on blots. The accurate information of the total protein in each lane on the blot, in combination with the signal information of the antibody of interest, makes it possible to precisely analyze the protein expression data. In addition, all fluorescent signals on the blot remain stable up to one year.

The T-West™ combined kit contains T-Dye protein label, bi-fluorescent protein marker, fluorescent secondary antibody, low fluorescence blotting membranes.

Key features

- Imaging of proteins and corresponding blots within seconds
- Wide dynamic range of total protein and antibody signals
- Up to 3 different antibody signals per blot
- Precise protein expression data analysis
- Signal stability up to 12 months

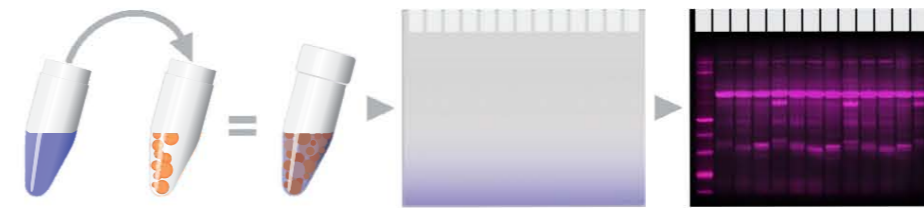
T-West™ Workflow

1 Label sample with fluorescent T-Dye.



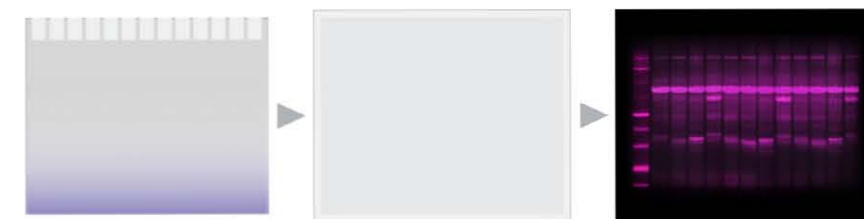
Hands-on time 5 min
Total time 30 min

2 Run SDS-PAGE and image gel.



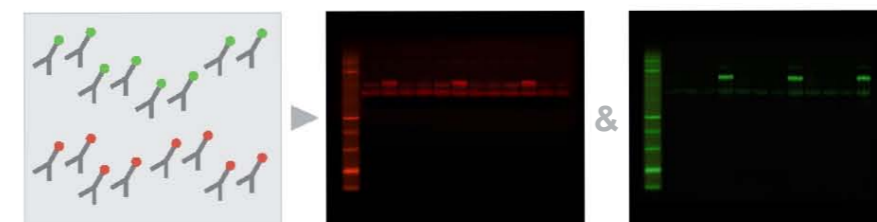
Sensitivity: 10 - 0.1ng
Dynamic range: 10^4 - 10^5
Exposure time: 1-2 sec

3 Transfer proteins and image blot.



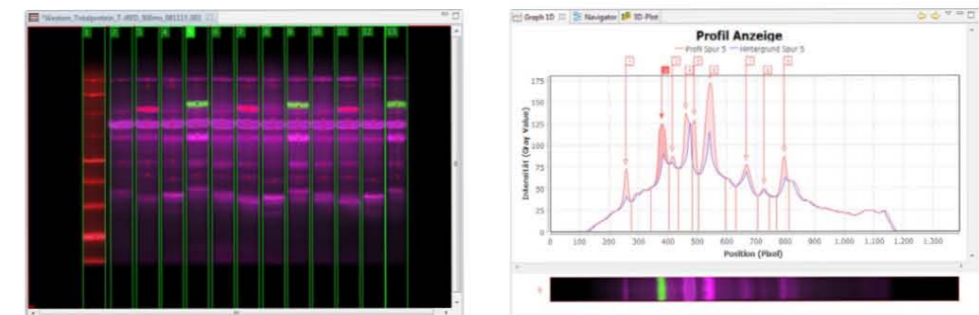
Sensitivity: 10 - 0.1ng
Dynamic range: 10^4 - 10^5
Exposure time: 0.1 - 1 sec
Signal stability: 6 - 12 months

4 Probe membrane with T-Dye conjugated antibody and image fluorescent signal.



Sensitivity: Equal to ECL
Dynamic range: 10^4 - 10^5
Exposure time: approx. 10 sec
Signal stability: 6 - 12 months

5 Overlay images and analyze data of total protein and protein of interest.



Fluorescent T-Rex protein label for 2D SDS-PAGE

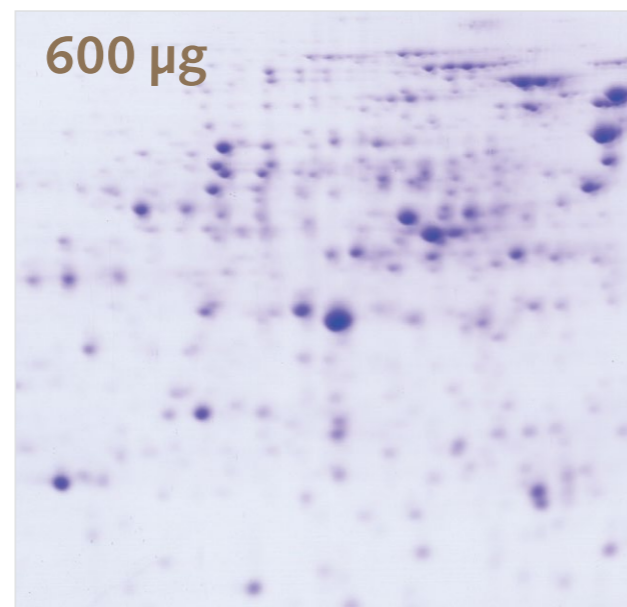
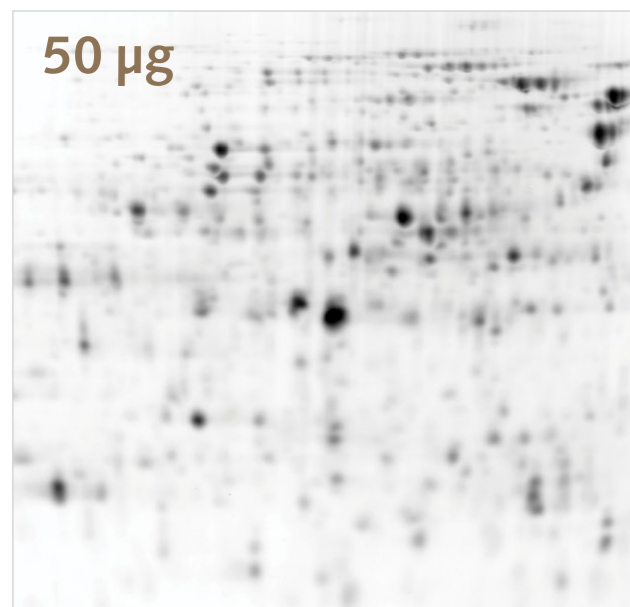
Are you searching a more sensitive, better reproducible, and timesaving replacement for post-stains? The powerful T-Rex protein label combines excellent performance and photostability over a wide pH-range of 2-11. Ideally suited for high performance fluorescent labeling of protein samples for 2D gels, T-Rex allow fast and easy protein visualization. In addition, T-Rex labeled proteins are perfectly detectable after transfer onto Western blot membranes and do not interfere with antibody recognition and mass spectrometry.

T-Rex label

- 50 µg protein sample
- 30 min total time for labeling
- Dynamic range 10^4 - 10^5

Coomassie® blue stain

- 600 µg protein sample
- 12 h total time for staining & destaining
- Dynamic range 10^2



T-Rex labeling vs. Coomassie® blue staining. 50 µg of protein derived from *E. coli* were labeled with T-Rex fluorescent label. Another 550 µg of unlabeled protein were added to the sample. The proteins were separated by 2D gel electrophoresis. T-Rex was detected with the OctoPlus QPLEX Fluorescence Imager by red epi fluorescence excitation. The gel was stained with Coomassie® blue and then imaged with the OctoPlus QPLEX Fluorescence Imager by white transmission light.

T-Rex protein label for 2D gels Characteristics



T-Rex

Detection sensitivity	0.05 ng *
Dynamic range	10^4 - 10^5
Photostability	+++
Suitable for WB **	+++
Excitation max.	650 nm
Emission max.	665 nm
Filter settings	G-Dye300, Cy5, Alexa 647
Kit content	<ul style="list-style-type: none"> • 20x T-Rex label (sufficient for 20 large gels) • T-Dye solvent

+++ Superior performance, * Sensitivity depending on imaging system, ** Western blot

Next generation gel-based top down proteomics

Refraction-2D™ | Saturn-2D™

Identify post-translational modifications ✓

Analyze up to 4 different samples per run ✓

Quantify protein expression ✓



"Analyzing samples with Refraction-2D™ allowed us to find post-translational protein modifications we would have missed by other approaches."

Dr. Christian Scharf, University of Greifswald

Refraction-2D™ QPLEX

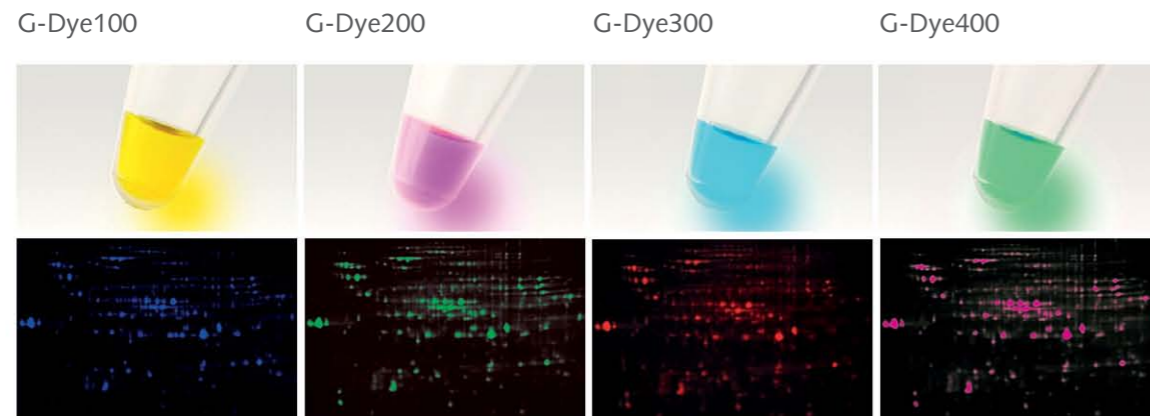
State of the art 2D gel based top down proteomics

Comparing up to 4 different samples within the same analysis: Refraction-2D™ QPLEX introduces the world's first 4-color coding for 2D gels. The G-Dyes (G-Dye100, G-Dye200, G-Dye300 and G-Dye400) combine the most powerful fluorescence properties with a superior photostability and easy, accurate spot picking.

New G-Dyes

Sensitivity up to 0.03 ng

- Perfect spot matching with RF-2D technique
- Superior sensitivity compared to Cyanine dyes
- 4-5 orders of magnitude dynamic range
- Unmatched photostability
- Accurate spot picking - no further staining required
- Quality kit assurance - diagnostic grade level

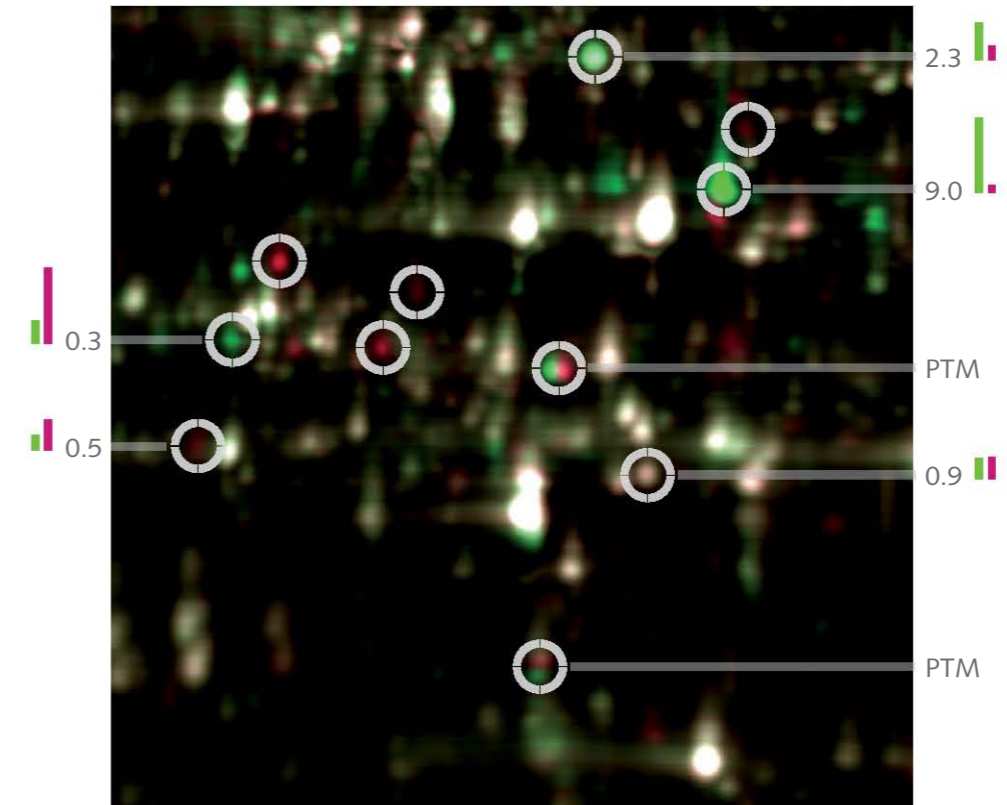


Imaging properties

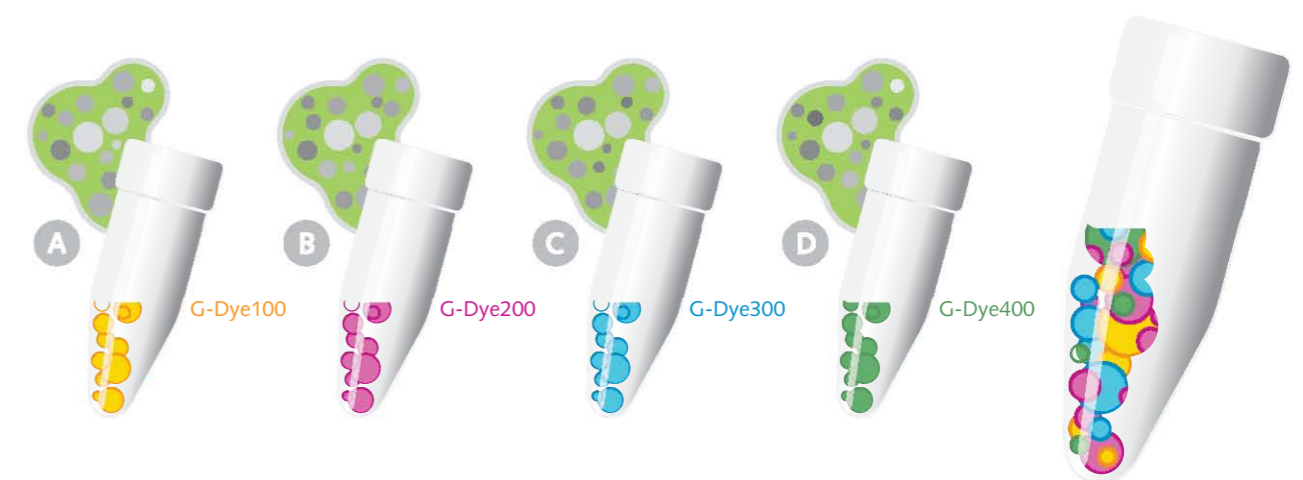
G-Dye	Fluorescence	Excitation max.	Emission max.
G-Dye100	blue	500 nm	510 nm
G-Dye200	green	550 nm	550 nm
G-Dye300	red	650 nm	660 nm
G-Dye400	infra red	736 nm	760 nm

Perfect multiplexing

Run and analyze different samples within one gel



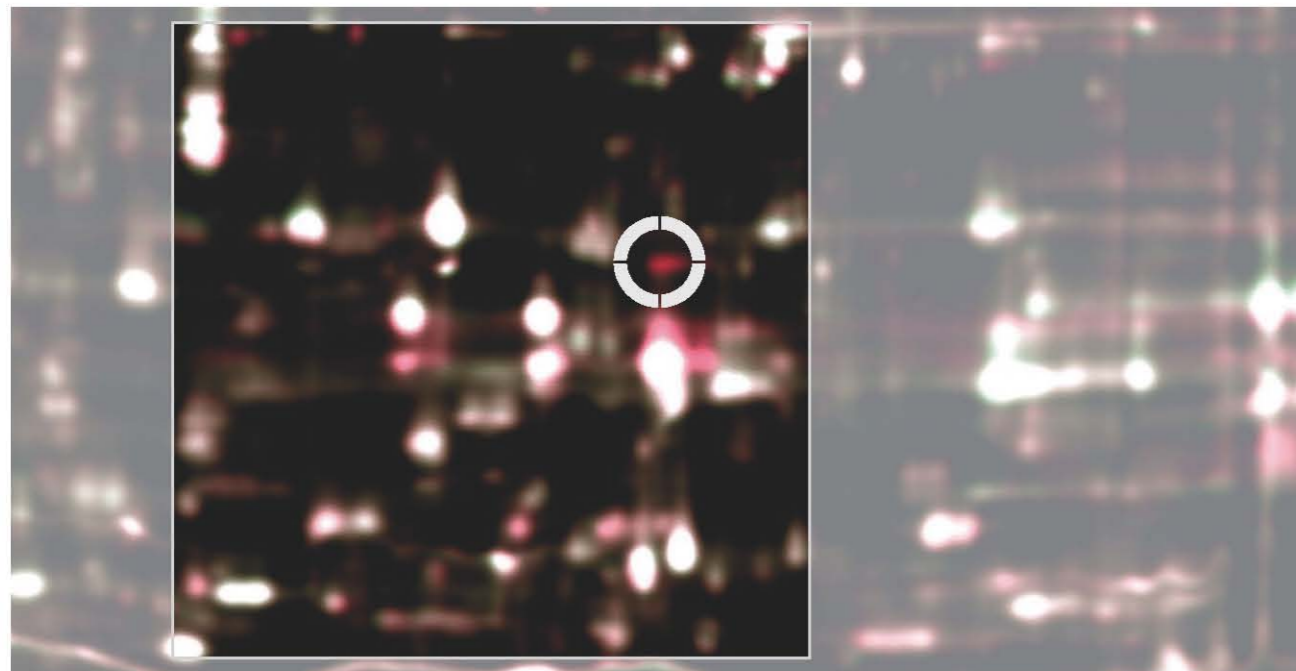
Refraction-2D™ analysis of *Arabidopsis thaliana* upon salt stress. 50 µg of protein derived from leaves of treated and untreated plants were labeled with G-Dye200 and G-Dye300 respectively. 25 µg of protein from each sample was labeled with G-Dye100 (internal standard). Proteins were separated by 2D SDS-PAGE and the G-Dye labels were detected by fluorescence imaging. Data analysis and evaluation was performed by 2D software. Indicated protein spots show the differences in the protein expression of the two samples (e.g. 0.5 = 2fold downregulation) or post translational modification (PTM).



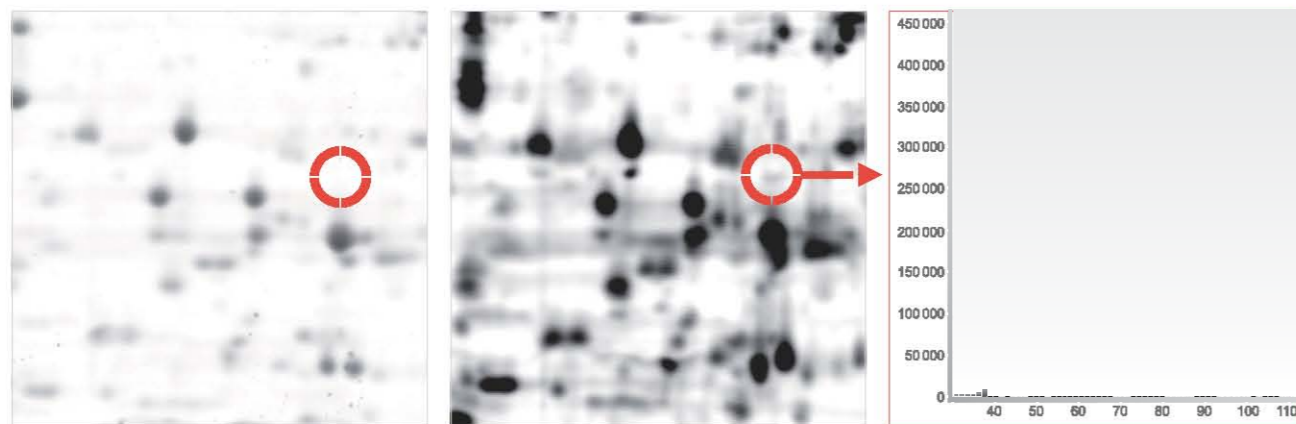
Accurate & easy spot picking

Identify and isolate the candidate proteins

The very low weight G-Dye100 fluorescent label comigrates perfectly with unlabeled protein in 2D gels. This allows for the accurate isolation of candidate proteins either automatically or manually without any further staining. All Refraction-2D™ kits come with an additional vial of G-Dye100 for accurate and easy spot picking.



Refraction-2D™ analysis of *Aspergillus fumigatus* upon iron stress. Size of indicated protein approx. 17 kDa.



Coomassie® blue stain

G-Dye100 labeling

MALDI-TOF analysis

Refraction-2D™ labeling kit content

Ready to use & matching your needs

Refraction-2D™ QPLEX labeling kits consists of the four G-Dyes (G-Dye100, G-Dye200, G-Dye300, G-Dye400), the Refraction-2D™ labeling kit of the three G-Dyes (G-Dye100, G-Dye200, G-Dye300). Each kit contains G-Dye solvent, a labeling stop solution and low retention pipette tips and tubes (MS compatible). Kit sizes 12G come with an extra vial of G-Dye100 for easy spot picking. All Refraction-2D™ and Refraction-2D™ QPLEX kits are subject to a strict quality control at diagnostic grade level.

- ✓ Higher sensitivity & photostability
- ✓ Accurate spot picking
- ✓ Free expert coaching
- ✓ Quality assured
- ✓ More usable



Saturn-2D™

Labeling for scarce samples

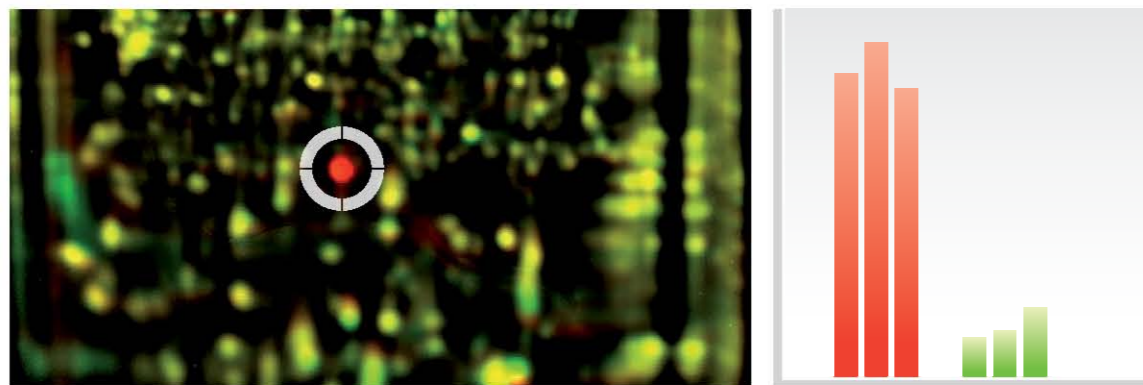
Saturn-2D™ is a novel technology for protein labeling of scarce samples using 2D gel based top down proteomics. Samples with as little as 5 µg protein can be analyzed. By labeling with a set of pre-eminent powerful fluorescent S-Dyes activated for binding to cysteine residues of proteins Saturn-2D™ allows a protein detection of up to 0.003 ng.

The Saturn-2D™ kit is ready to use containing the S-Dyes, S-Dye solvent, TCEP for cysteine reduction, sterile H₂O, as well as S-Dye low retention tips and micro centrifuge tubes.

Powerful S-Dye cysteine labeling

Sensitivity up to 0.003 ng

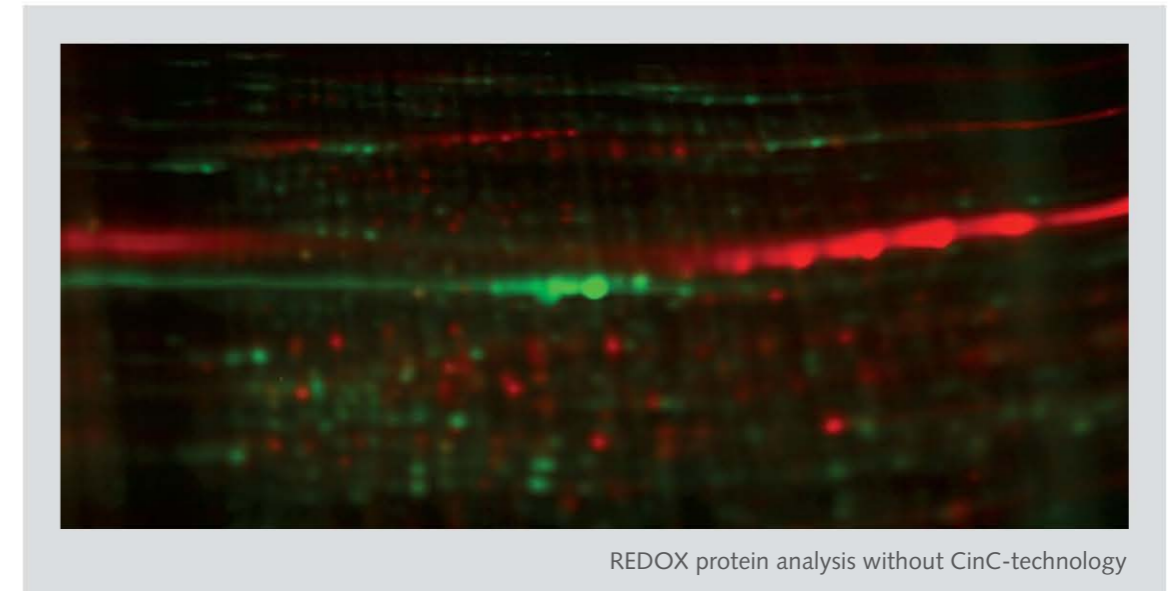
- Superior sensitivity compared to Cyanine dyes
- Dynamic range: 4-5 orders of magnitude
- Unmatched photostability
- Mass spec compatible



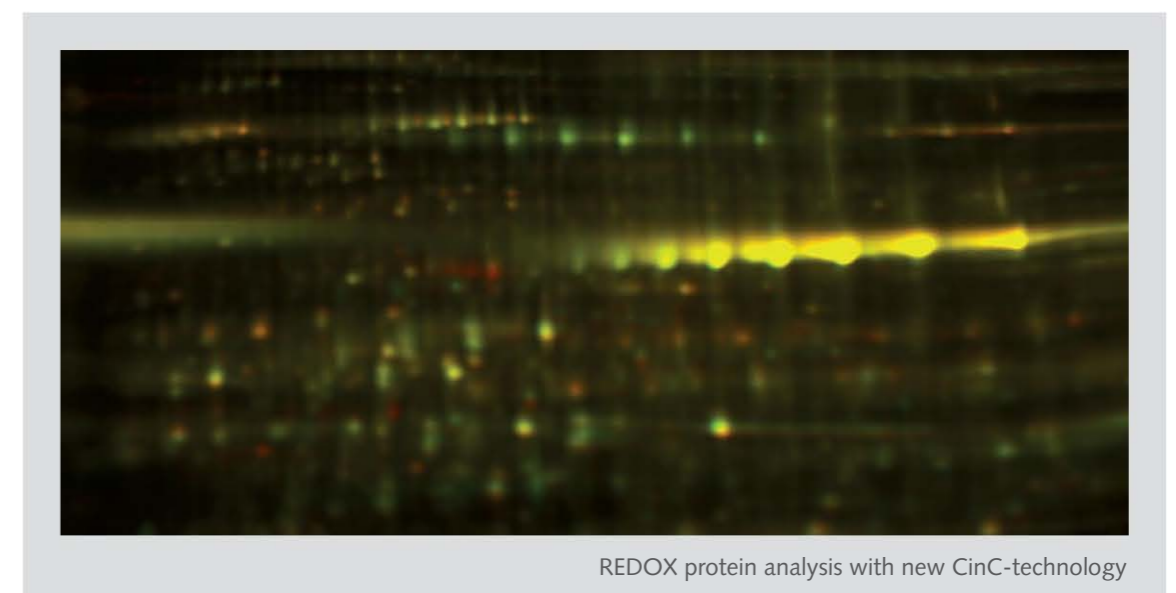
Saturn-2D™ REDOX

Stress response and cellular signaling at a glance

Stress response and cellular signaling is manifested in multiple shifts in the proteome by oxidation of Cys residues. The new Saturn-2D™ REDOX protein labeling kit perfectly illustrates changes within the complex redox interaction network. Due to specially designed Cys-interacting compounds (CinC) differences in the redox status can be easily displayed.



2D gel analysis using traditional multiplex fluorescence labeling kits. Unequal binding of dyes to Cys-residues to reduced and oxidized proteins leads to inconsistent spot matching.



2D gel analysis using CinC-technology. Proteins differing in their REDOX status show perfect spot matching. Differences in the REDOX potential are displayed in changes of fluorescence intensities.

Saturn-2D™ REDOX

Perfect spot matching with CinC™-technology

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 is solved by a specially designed Cys-interacting compound (CinC).

For further technical information please refer to our detailed Saturn-2D™ REDOX Product Guide.

Using CinC™-technology (e.g. non-direct approach)

Protein A (unstressed)



Protein B (stressed)

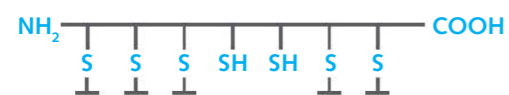


1. Blocking of reduced Cysteines by Cys-interacting compound (CinC)



2. Removal of excessive Cys-interacting compound (CinC)

3. Reduction of oxidized Cysteines



4. Labeling of oxidized Cysteines with S-Dyes



Saturn-2D™ labeling

Kit content

- S-Dye200 – high performance fluorescence dye
- S-Dye300 – high performance fluorescence dye
- S-Dye solvent
- ddH₂O, sterile
- TCEP
- S-Dye low retention centrifuge tubes
- S-Dye low retention tips



Saturn-2D™ REDOX labeling

Kit content

Pack 1

- S-Dye200 – high performance fluorescence dye
- S-Dye300 – high performance fluorescence dye
- S-Dye solvent
- CinC /CinC solvent
- Redox labeling buffer
- Redox stop solution
- ddH₂O, sterile
- TCEP

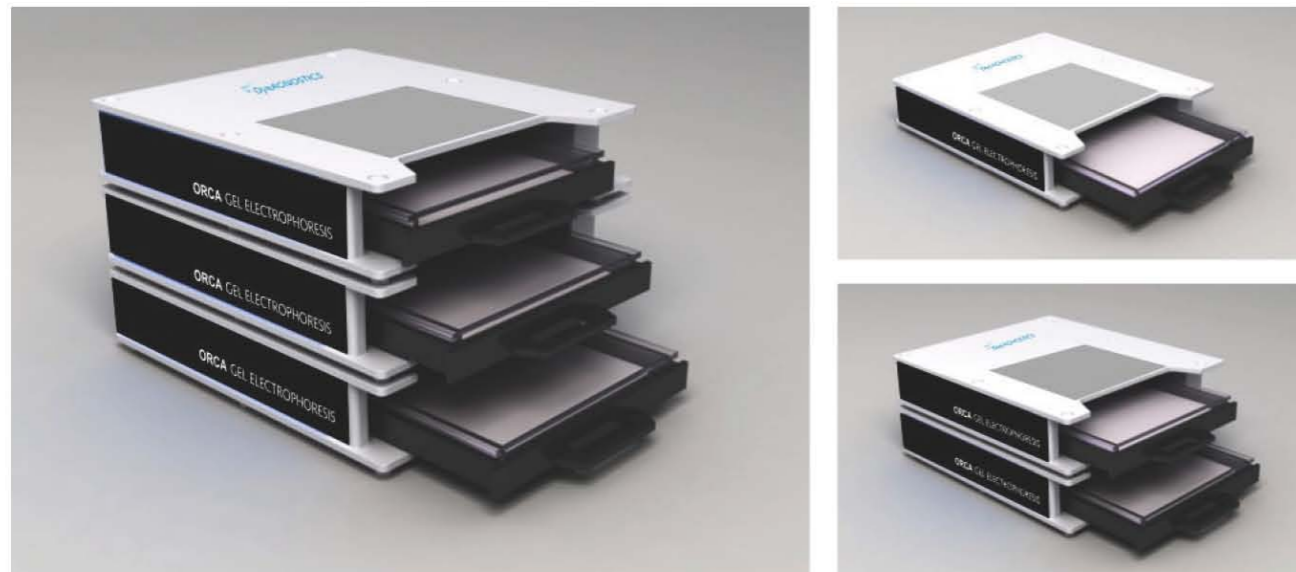
Pack 2

- S-Dye low retention tips
- S-Dye low retention tubes
- Redox wash buffer
- Spin columns
- Redox matrix

ORCA Gel Electrophoresis

Modular horizontal protein separation system

The Orca system introduces new modular gel electrophoresis units and novel Velum™ precast gels. This combination offers best 1D and 2D SDS-PAGE results in terms of resolution and reproducibility. Compared to other horizontal protein separation systems, Orca provides a unique array of highly robust electrodes, which allows to run two 1D Velum™ gels onto a single Orca unit at the same time. The gel system is easy to use and up to three combined Orca systems run simultaneously using the same power supply and cooling unit.



Key features

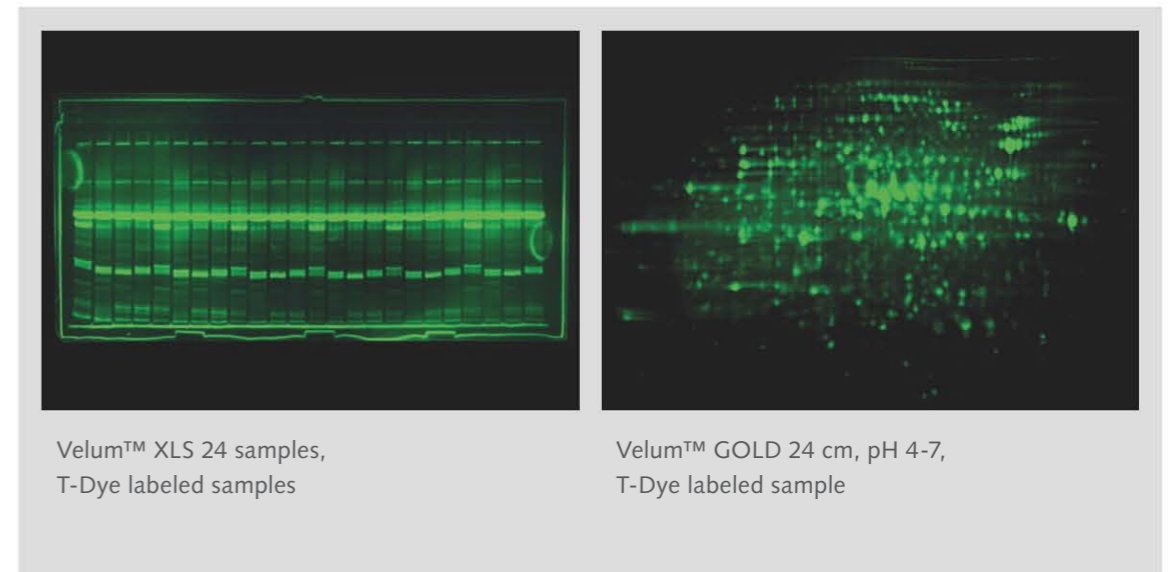
- Easy to use
- Powerful protein separation
- Combination of three units possible
- Unique array of robust electrodes for dual runs



VELUM™ Precast Gels

Thin film-backed gels for IEF, 1D- and 2D-SDS-PAGE

Velum™ precast gels offer perfect protein separation and a novel experience of convenience in handling. No glass plates and very small amounts of buffer (included with the kit) are required. For 1D SDS-PAGE up to 52 samples can be separated on one gel. Using the Orca Gel Electrophoresis unit, 2 Velum™ XLS gels can be run at the same time. Velum™ XLS and Velum™ SILVER gels are ideally suited for red and IR-fluorescence protein imaging with a tenfold better signal to noise ratio compared to other gels.



Key features

- High reproducibility
- Perfect electrophoretic protein separation
- Allows to separate up to 104 samples per 1D run
- Most convenient handling for imaging and blotting



BEO Dry Blotter

Simply driven for a phenomenal quality of transfer

Sometimes it may not be the time that matters but the result. The Beo Dry Blotter transfers protein simply based on capillary action with phenomenal results in terms of transfer quality and efficiency. The blotting does neither require power nor additional buffer. And you can process up to 18 blots derived from mini gels or 3 blots from large gels (e.g. Velum™ 1D, IEF or 2D gels) at the same time.



Key features

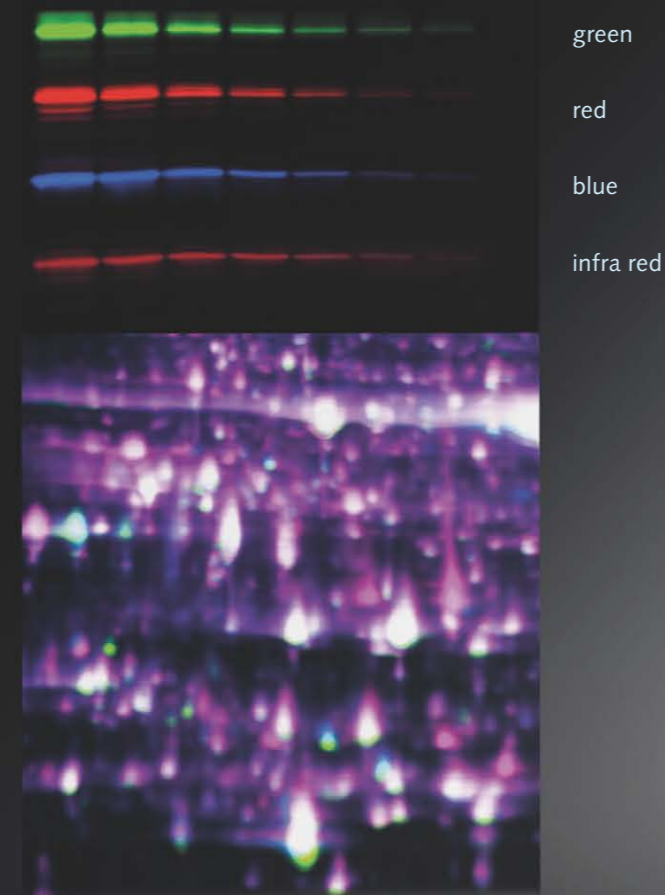
- Easy to use
- High quality of protein transfer
- No additional buffers, requires no power
- Takes up to 18 blots from mini gels or 3 large blots from 2D gels



Octopus QPLEX Fluorescence Imager

Sensitive image acquisition within seconds

- Highly specific 4-color fluorescence imaging
- Large imaging for e.g. 2D-gels and -blots
- 4-5 orders of magnitude dynamic range
- Robust system for daily usage



green

red

blue

infra red



Product information

T-West™ combined kit

Prod. No.	Description
PR883	T-West combined kit for 10 mini gels containing 1x Bi-fluorescent protein marker (50 µl) 1x Blotting kit PR811 1x Fluorescence T-Dye protein label PR801 or PR803 or PR805 1x Fluorescent secondary antibody PR831 or PR832 or PR833 or PR834
PR884	T-West combined kit for 4 Velum gels containing 1x Bi-fluorescent protein marker (50 µl) 1x Blotting kit PR812 1x Fluorescence T-Dye protein label PR801 or PR803 or PR805 1x Fluorescent secondary antibody PR831 or PR832 or PR833 or PR834
Other sizes on request	

Fluorescent T-Dye protein labeling kit

Prod. No.	Description
PR801	Green-fluorescence sample labeling 100 rcts. (e.g. for 10 mini gels)
PR802	Green-fluorescence sample labeling 200 rcts. (e.g. for 20 mini gels)
PR803	Red-fluorescence sample labeling 100 rcts. (e.g. for 10 mini gels)
PR804	Red-fluorescence sample labeling 200 rcts. (e.g. for 20 mini gels)
PR805	IR-fluorescence sample labeling 100 rcts. (e.g. for 10 mini gels)
PR806	IR-fluorescence sample labeling 200 rcts. (e.g. for 20 mini gels)

Fluorescent protein marker

Prod. No.	Description
PR821	Bi-fluorescent (red & green) protein marker 500 µl (e.g. for 100 lanes)
PR822	Tri-fluorescent protein marker (red & green & IR), 500 µl (e.g. for 100 lanes), product on request
Other combinations on request	

Fluorescent secondary antibody

Prod. No.	Description
PR831	Green-fluorescent anti-mouse IgG for 10 mini gels or 4 Velum gels
PR832	Green-fluorescent anti-rabbit IgG for 10 mini gels or 4 Velum gels
PR833	Green-fluorescent anti-goat IgG for 10 mini gels or 4 Velum gels
PR834	Green-fluorescent anti-human IgG for 10 mini gels or 4 Velum gels
Other colors or species on request	

Blotting kit

Prod. No.	Description
PR811	Low-fluorescence membrane + blotting paper, precut for 10 mini gels size 100 x 100 mm
PR812	Low-fluorescence membrane + blotting, precut for 4 Velum gels size 250 x 120 mm
Other sizes on request	

Refraction-2D™ labeling kit 3-color protein labeling (blue, green, red)

Prod. No.	Description
PR08	Refraction-2D™ labeling kit, size 4G
PR08G	Refraction-2D™ labeling kit, size 8G
PR09	Refraction-2D™ labeling kit, size 12G
PR11	Refraction-2D™ labeling kit, size 24G

PR12	Refraction-2D™ labeling kit size 36G
PR13	Refraction-2D™ labeling kit size 72G
PR014	Refraction-2D™ spot picking kit size 3G

Refraction-2D™ QPLEX labeling kit 4-color protein labeling (blue, green, red, IR)

Prod. No.	Description
PR60	Refraction-2D™ QPLEX labeling kit size 4G
PR61	Refraction-2D™ QPLEX labeling kit size 8G
PR62	Refraction-2D™ QPLEX labeling kit size 12G

Saturn-2D™ labeling kit

Prod. No.	Description
PR30	Saturn-2D™ titration kit size XS
PR31	Saturn-2D™ labeling kit size 4S
PR32	Saturn-2D™ labeling kit size 8S
PR32S	Saturn-2D™ preparative kit size 8S + 40S

Saturn-2D™ REDOX labeling kit

Prod. No.	Description
PR34	Saturn-2D™ REDOX protein labeling kit, size 8S
PR35	Saturn-2D™ REDOX protein labeling kit, size 12S

1D SDS-PAGE and IEF precast gel

Prod. No.	Description
PR227	Velum XLS for 24 samples, 12.5% AA, pack of 4
PR223	Velum XLS for 52 samples, 12.5% AA, pack of 4
PR224	Velum IEF for 24 samples, pH on request, pack of 4
PR228	Velum IEF for 40 samples, pH on request, pack of 4
PR229	Velum IEF for 80 samples, pH on request, pack of 4

2D SDS-PAGE precast gel

Prod. No.	Description
PR221	Velum SILVER 2x 11 cm, pH 4-7 for green, red and IR fluorescence Pack of 4
PR222	Velum SILVER for 1x 24 cm, pH 4-7 for green, red and IR fluorescence Pack of 4
PR225	Velum GOLD for 1x 24 cm, pH 4-7 for RGB+IR Fluorescence Pack of 4, Product on request

Protein separation

Prod. No.	Description
PR201	Orca Gel Electrohoresis unit

Protein transfer

Prod. No.	Description
PR87	Beo Dry Blotter including 2 separation sheets

Image acquisition

Prod. No.	Description
PR130	Octoplus QPLEX Fluorescence Imager

Data analysis software

Prod. No.	Description
PR136	LabImage 1D Automatic 1D gel and Western blot evaluation software
PR134	Delta2D State of the art 2D analysis software fix, floating, consumable

Contact

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