

2012-2013

HUNTING FOR CANDIDATE PROTEINS

Protein labeling kits for modern gel based top down proteomics

Modern gel based top down proteomics:

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

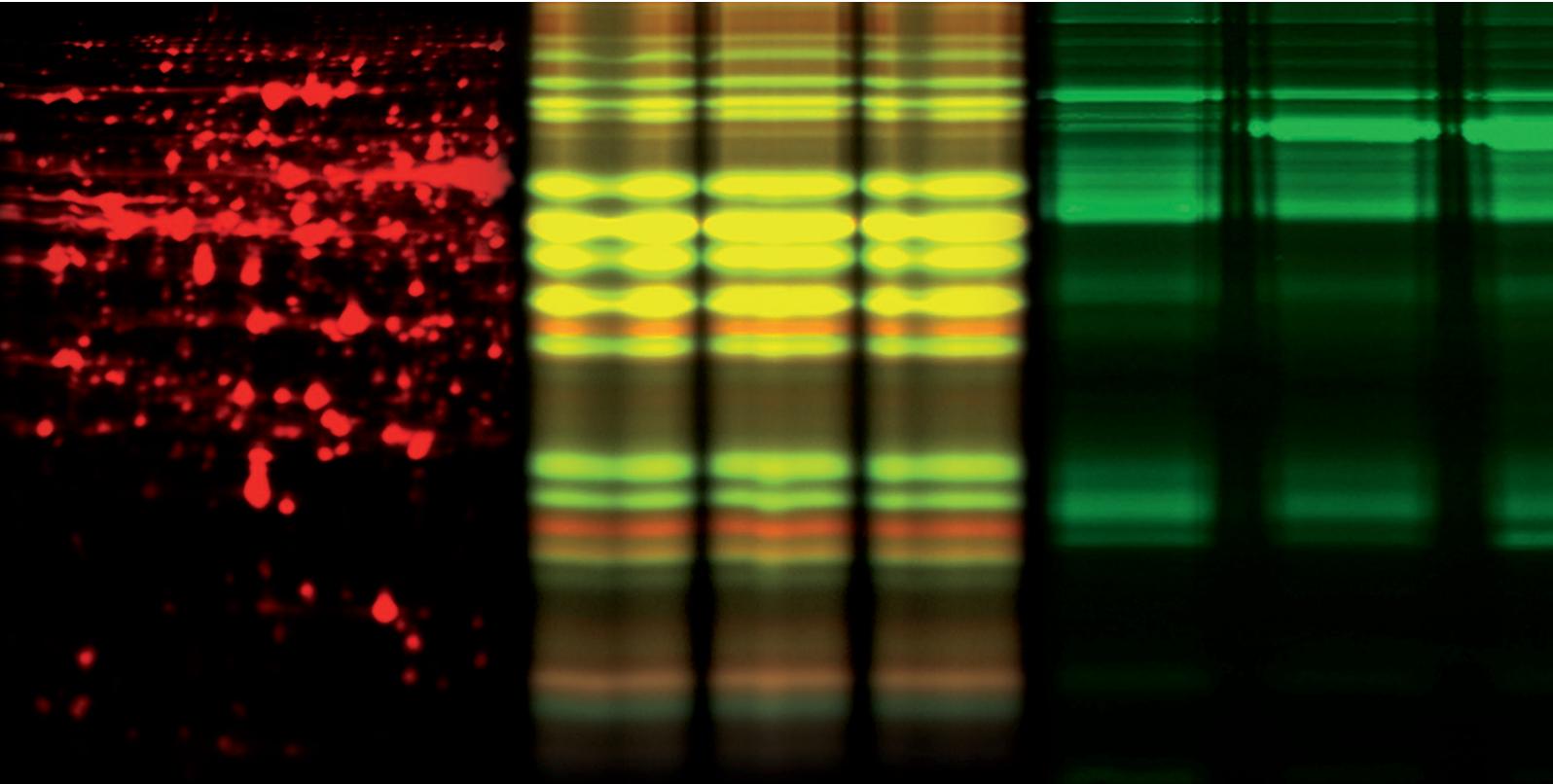
Dr. Christian Scharf, University of Greifswald

Fluorescence labeling of proteins

T-Dyes Series

- 1-2-3 easy protein labeling
- 20 single reactions per kit
- Excellent performance

T-Green 210 | T-Red 310 | T-Rex 330



**IDEAL REPLACEMENT
OF POST STAINS**

**COMPARE DIFFERENT
SAMPLES IN ONE WELL**

**SAVE SAMPLE AND
TIME FOR VISUALIZATION**

“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

T-Dye series

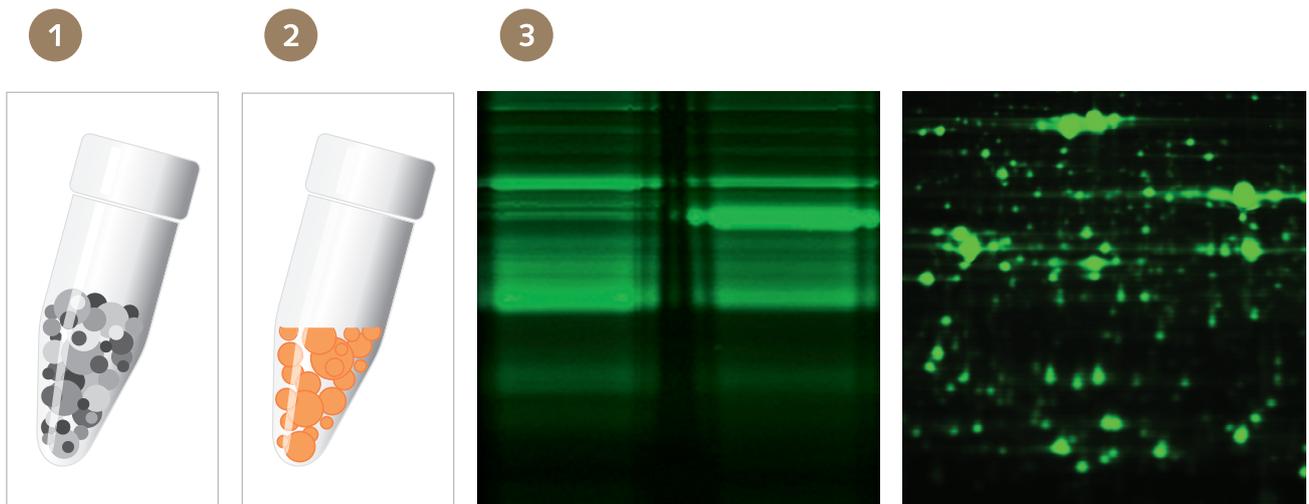
Ideal replacement for post-stains, sample saving

The novel T-Dye family combines excellent fluorescence properties with an easy 5 min hands-on protein labeling. The T-Dyes are ideal for sensitive protein visualization in 1D and 2D SDS-PAGE gels: highly sensitive, time saving and with best reproducibility. All T-Dye kits come with 20 single aliquots, each sufficient for labeling of 50 μ g of protein.

T-Dye series

1-2-3: Easy & fast protein labeling

- 1. Extract proteins in compatible buffer**
(for more details please check out the T-Dye product guide)
- 2. Label proteins with T-Dyes**
 - Dissolve T-Dye in T-Dye solvent
 - Add protein and incubate for 30 min on ice
 - Quench labeling by adding stop solution
 - Sample is ready for 1D/2D PAGE
- 3. Detect label by fluorescence imaging**



1D SDS-PAGE: 1 μ g sample per lane 2D SDS-PAGE 50 μ g sample per gel

T-Dye series

for 1D SDS-PAGE

Fast and easy T-Dye labeling allows you to analyze as little as 1-2 μg of protein sample per lane with accurate detection and quantification. Specific fluorescent labels allow the the combination of different samples within one analysis (sample multiplexing). Western Blot protein transfer can be easily visualized by fluorescent imaging.

1D SDS-PAGE Save sample and time

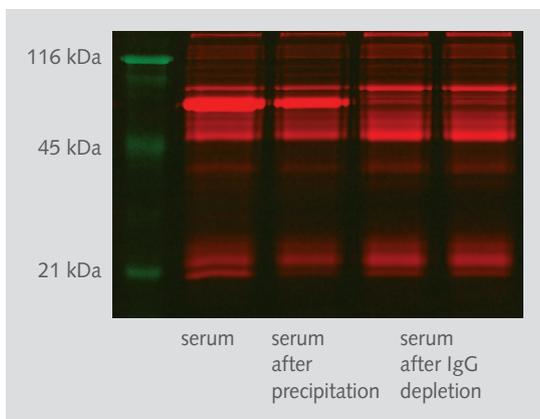


Fig. 1. T-Green 210 labeled human serum protein (1 μg per lane).

Western Blotting Visualize protein transfer

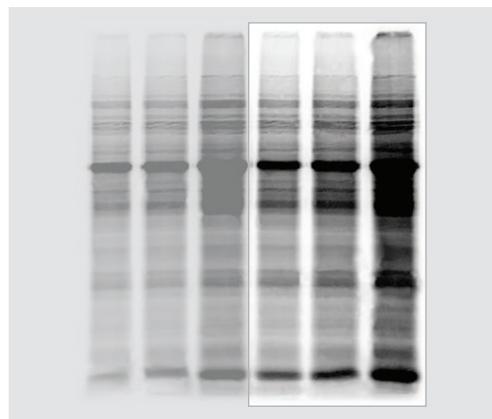


Fig. 2. Western blot with T-Red 310. Lane 4: 1 μg , lane 5: 2 μg ; lane 6: 5 μg of protein derived from *Arabidopsis thaliana*.

Sample Multiplexing Analyze different samples in a single lane

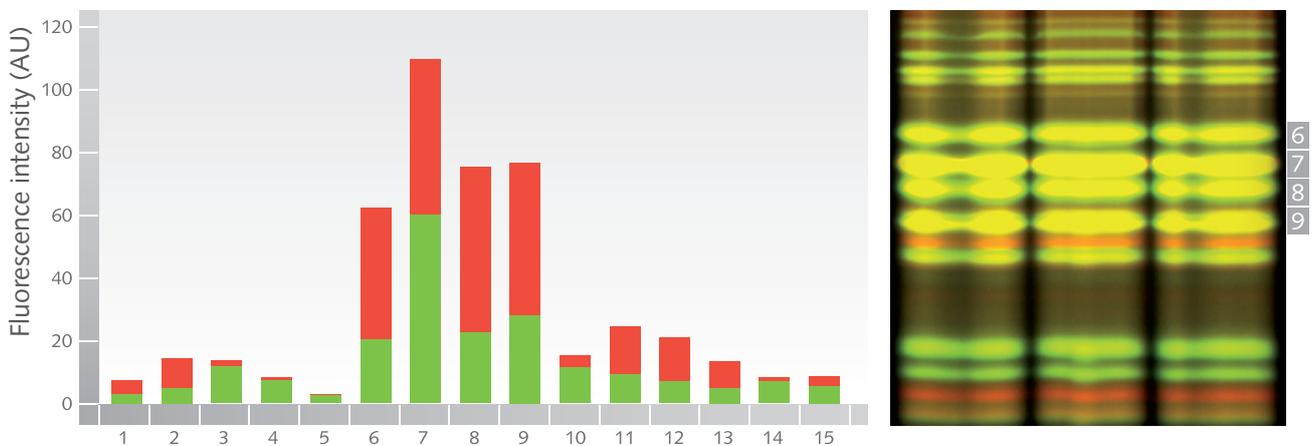


Fig. 3. Sample multiplexing of 2 μg T-Red 310 and T-Green 210 labeled protein derived from domestic pig (*Sus scrofa domesticus*) comparing two different extraction protocols. Evaluation by 1D software.

T-Dye series

for 2D SDS-PAGE

Are you searching a more sensitive, better reproducible, and timesaving replacement for post-stains? The T-Red 310 and the even more powerful T-Rex 330 combine 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-Red 310 and T-Rex 330 allow fast and easy protein visualization.

T-Rex 330 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

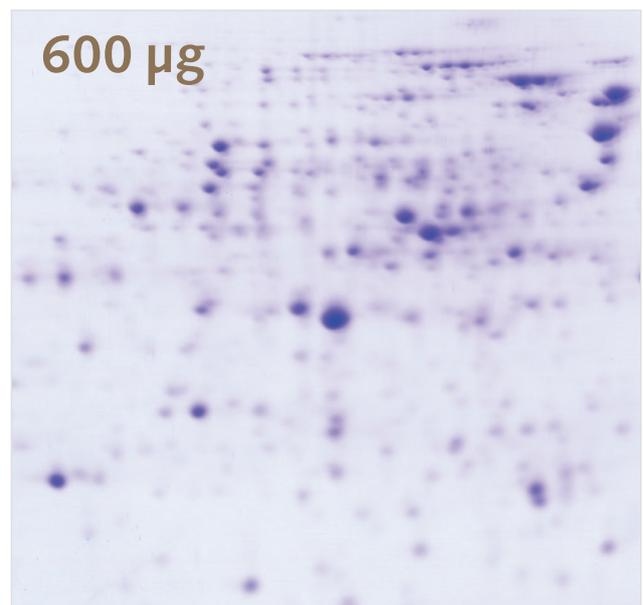
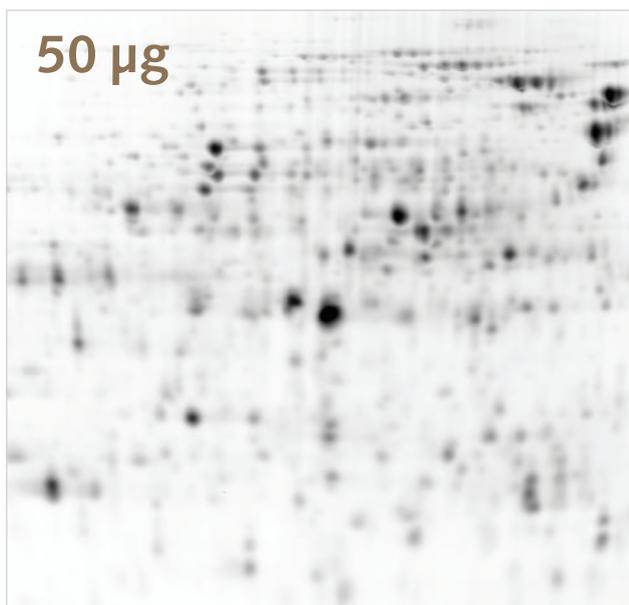


Fig. 4. T-Rex 330 labeling vs. Coomassie® blue staining. 50 µg of protein derived from *E. coli* were labeled with T-Rex 330 fluorescent dye. Another 550 µg of unlabeled protein were added to the sample. The proteins were separated by 2D gel electrophoresis. T-Rex 330 was detected with the OCTOPLUS fluorescence imager by red epi fluorescence excitation. The gel was stained with Coomassie® blue and then scanned with the OCTOPLUS fluorescence imager by white transmission light.

T-Dye protein labeling kits

Characteristics

| | T-Green 210 | T-Red 310 | T-Rex 330 |
|-----------------------|---|---|---|
| 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 1D/ 2D | 1D +++ / 2D ++ | +++ | +++ |
| Suitable for WB | +++ | +++ | +++ |
| Excitation max. | 559 nm | 650 nm | 650 nm |
| Emission max. | 585 nm | 665 nm | 665 nm |
| Filter settings | G-Dye200, Cy3, Alexa 555 | G-Dye300, Cy5, Alexa 647 | G-Dyes300, Cy5, Alexa 647 |
| Kit content | 20 x T-Green 210 T-Dye solvent T-Dye labeling stop sol. | 20 x T-Red 310 T-Dye solvent T-Dye labeling stop sol. | 20 x T-Rex 330 T-Dye solvent T-Dye labeling stop sol. |

++ High performance +++ Superior performance

* Sensitivity depending on imaging system



Next generation sample multiplexing

Refraction-2D™ | Saturn-2D™

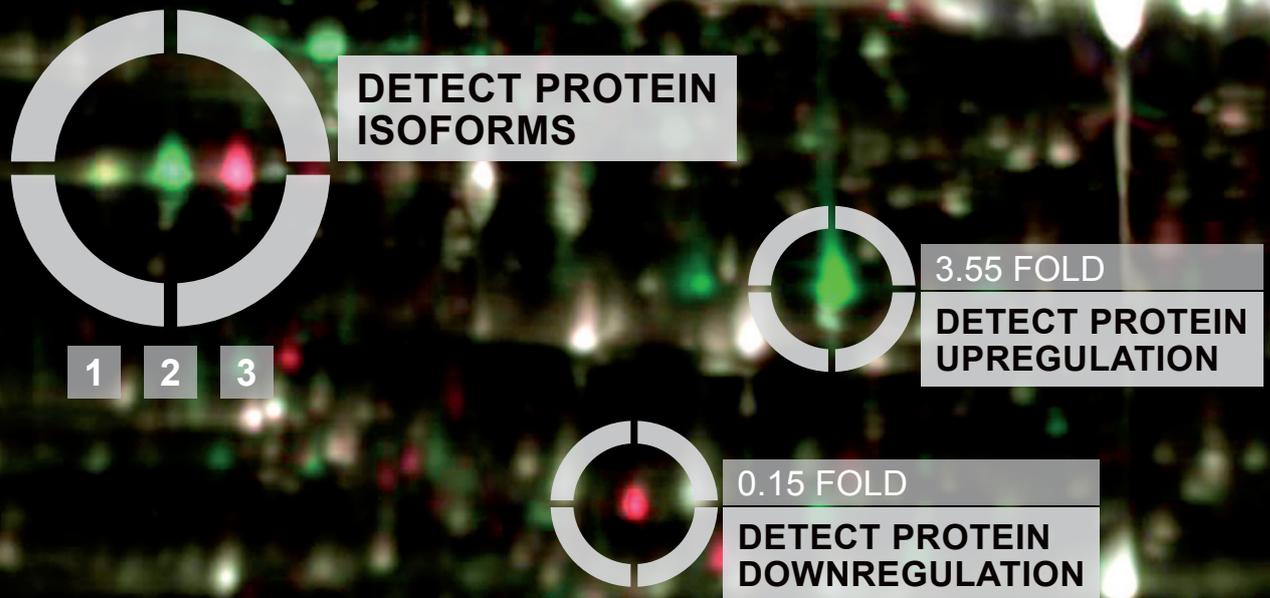
Unmatched sensitivity

Accurate quantitation

Easy spot picking

Refraction-2D™

Saturn-2D™



“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™

Novel technology for 2D gel based top down proteomics

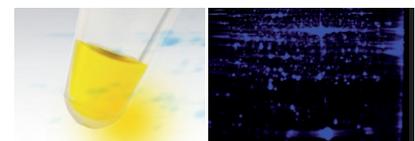
Refraction-2D™ combines the unmatched separation capacity of 2D gel electrophoresis and the information of intact proteins, with the highly sensitive and photostable fluorescent labels of new powerful G-Dyes.

G-Dyes bind covalently to proteins as activated NHS-esters. A single fluorophore per protein allows the quantitative analysis and detection of proteins as low abundant as 0.03 ng. For the first time, fluorescent labeled proteins can be accurately isolated from the gel with no further staining required.

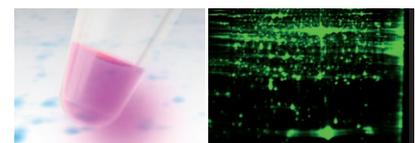
New G-Dyes

Sensitivity up to 0.03 ng

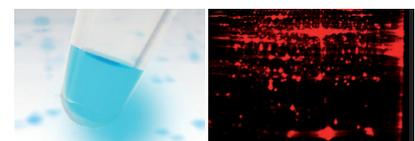
- Dynamic range: 4-5 orders of magnitude
- Non-interference of fluorescent light emission
- Accurate spot picking - no further staining required
- Quality assurance - diagnostic grade level
- Unmatched photostability
- Mass spec compatible



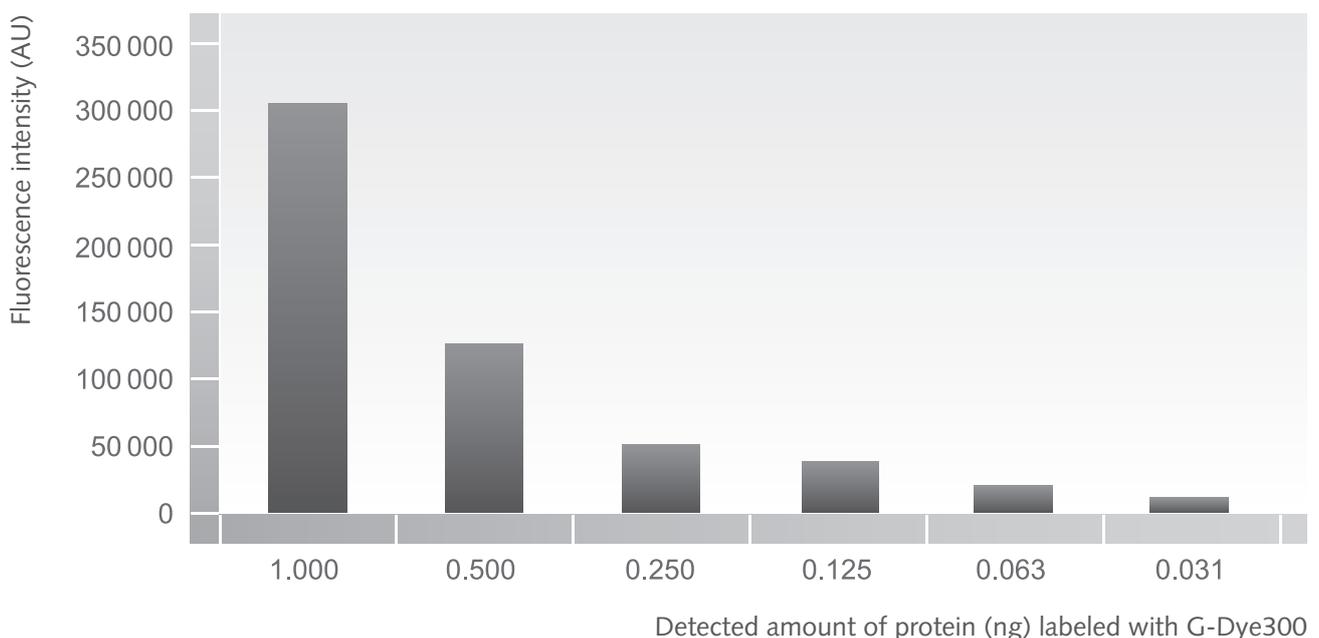
G-Dye100



G-Dye200



G-Dye300



Perfect multiplexing

Run and analyze different samples within one gel

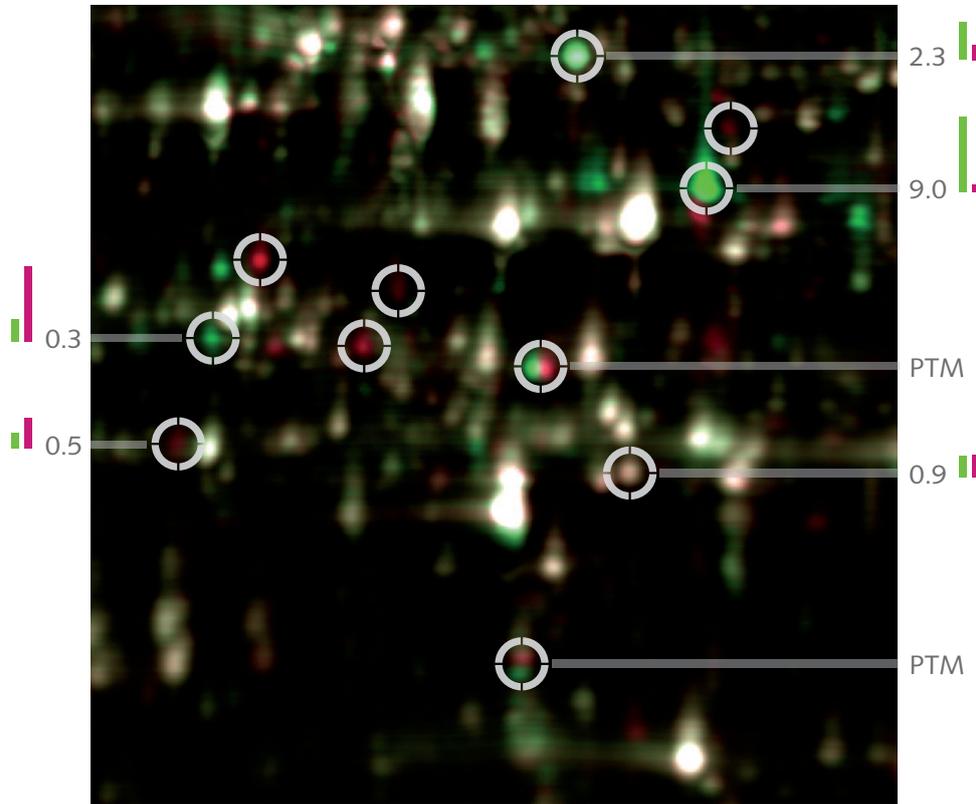
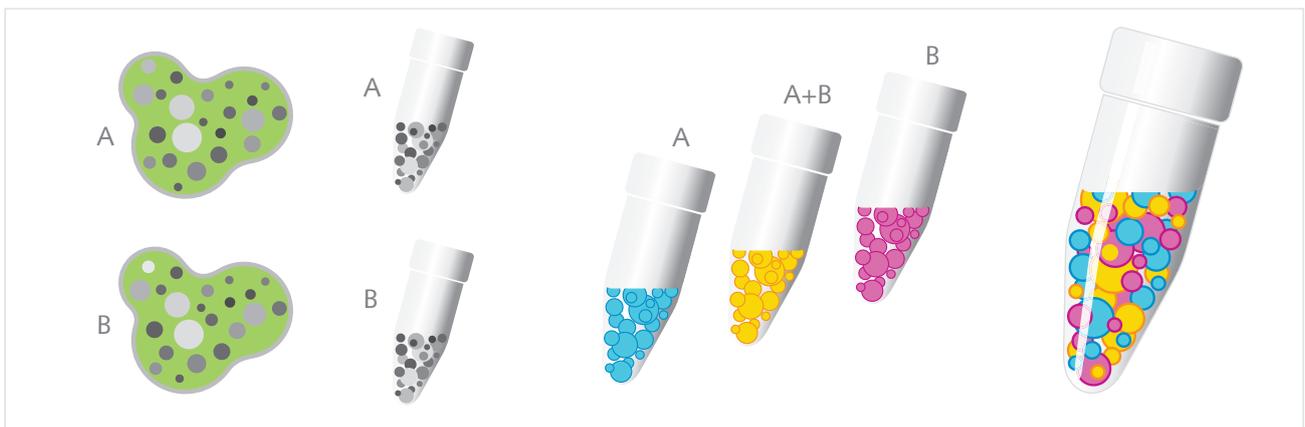


Fig. 5. Refraction-2D™ analysis of *Arabidopsis thaliana* upon salt stress. 50 µg of protein derived from leaves of treated and untreated plants were labeled with G-Dye200 and G-Dye300 respectively. 25 µg of protein from each sample was labeled with G-Dye 100 (internal standard). Proteins were separated 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 approximately up to the 5 kDa region. This allows for the accurate isolation of identified candidate proteins (fig. 6) either automatically or manually (fig. 7, 8, 9) without any further staining. Refraction-2D™ kits come with an additional vial of G-Dye100 for accurate and easy spot picking.

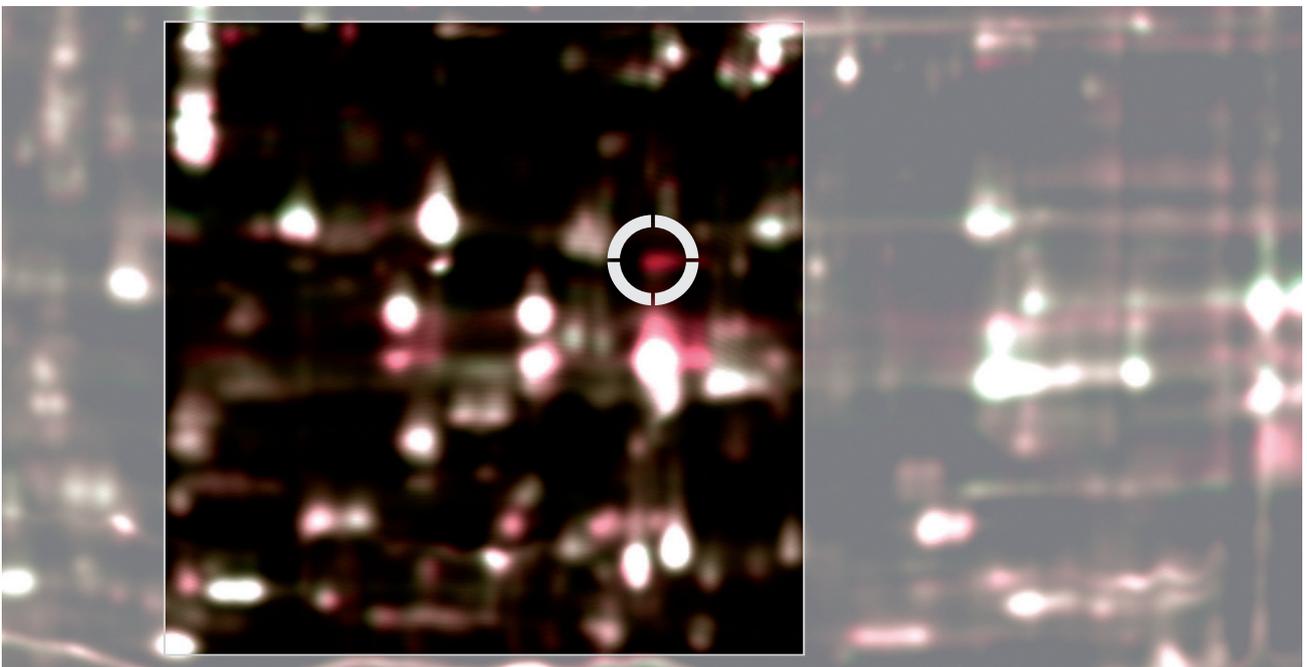


Fig. 6. Refraction-2D™ analysis of *Aspergillus fumigatus* upon iron stress. Size of indicated protein approx. 17 kDa.



Fig. 7. Coomassie® blue stain

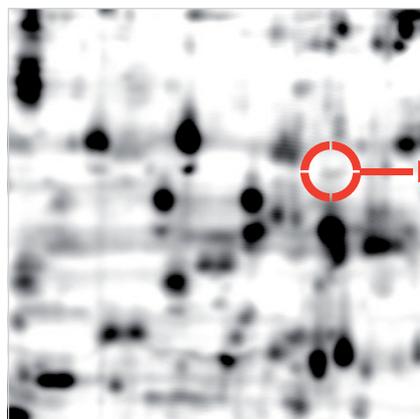


Fig. 8. G-Dye100 labeling

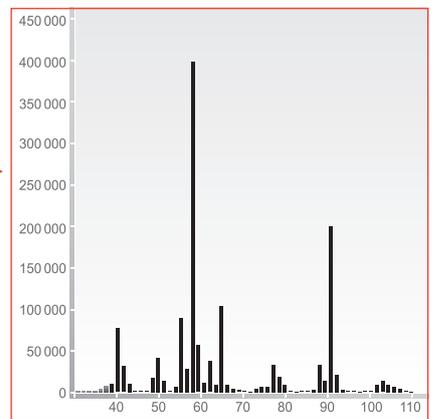


Fig. 9. MALDI-TOF analysis

Refraction-2D™ labeling kit content

Ready to use & matching your needs

- G-Dye100 – high performance fluorescence dye
- G-Dye200 – high performance fluorescence dye
- G-Dye300 – high performance fluorescence dye
- G-Dye labeling stop solution
- G-Dye solvent
- G-Dye low retention tips
- G-Dye low retention micro centrifuge tubes
- Extra G-Dye100 for easy spot picking (included in 12G kits and larger)

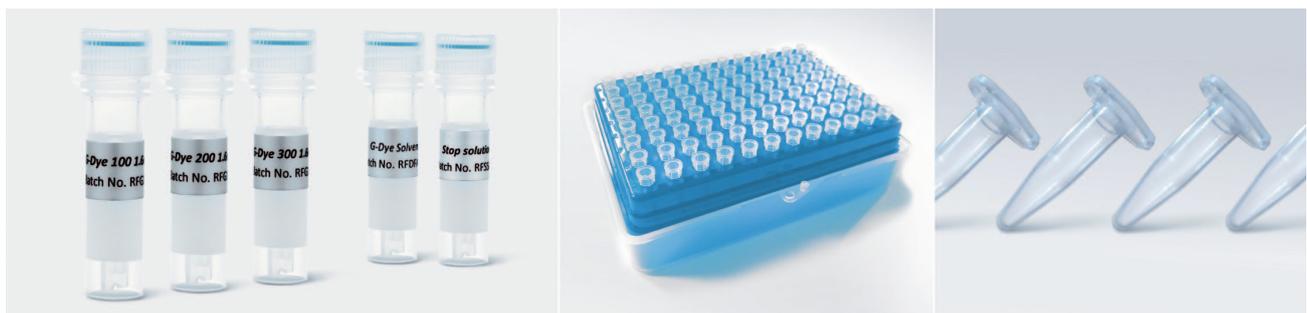
✓ **Higher** sensitivity & photostability

✓ **Accurate** spot picking

✓ **Free** expert coaching

✓ **Quality** assured

✓ **More** usable



Saturn-2D™

Powerful 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-Dye solvent, TCEP for cysteine reduction, sterile H₂O, as well as S-Dye low retention tips and micro centrifuge tubes.

New S-Dye cysteine labeling

Sensitivity up to 0.003 ng

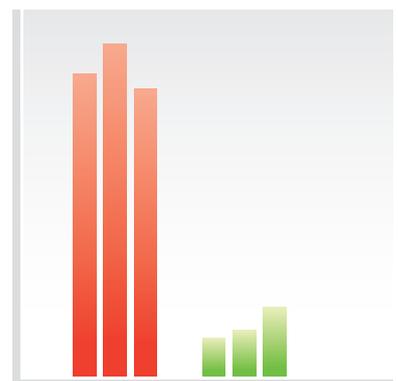
- Dynamic range: 4-5 orders of magnitude
- Quality assurance - diagnostic grade level
- Unmatched photostability
- Mass spec compatible



S-Dye300



S-Dye200



Saturn-2D™ labeling kit content

Ready to use & matching your needs

- S-Dye200 – high performance fluorescence dye
- S-Dye300 – high performance fluorescence dye
- S-Dye solvent
- ddH₂O, sterile
- TCEP (tris(2-carboxyethyl)phosphine)
- S-Dye low retention tips
- S-Dye low retention micro centrifuge tubes



Higher sensitivity & photostability



Free expert coaching



Quality assured



More usable



Refraction-2D™ or Saturn-2D™ for my sample?

Refraction-2D™

Saturn-2D™

Ideal protein concentration:
>5 $\mu\text{g } \mu\text{l}^{-1}$

Ideal protein concentration:
> 0.55 $\mu\text{g } \mu\text{l}^{-1}$

- Detection sensitivity: > 0.03 ng
- Protein amount: 50 μg per sample
- Linearity range: 10^4 - 10^5
- Sample multiplexing: Up to 3
- Sensitive quantification

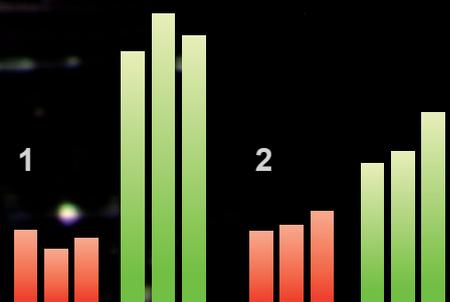
• **Application area:**

Sensitive minimal labeling
for proteome analyses

- Detection sensitivity: > 0.003 ng
- Protein amount: < 5 μg per sample
- Linearity range: 10^4 - 10^5
- Sample multiplexing: Up to 2
- Sensitive quantification

• **Application area:**

Sensitive saturation labeling
analyses of scarce protein samples
or REDOX proteomics



Ordering information

T-Dye protein labeling kits

| Prod. No. | Description | Kit size |
|-----------|------------------------------|----------|
| PR05 | T-Green 210 Labeling Kit | 20G |
| PR07 | T-Red 310 Labeling Kit | 20G |
| PR71 | T-Green + T-Red Labeling Kit | 20G |
| PR06 | T-Rex 330 Labeling Kit | 20G |

Refraction-2D™ protein labeling kits

| Prod. No. | Description | Kit size |
|-----------|---------------------------------|----------|
| PR08 | Refraction-2D™ Labeling Kit | 4G |
| PR08G | Refraction-2D™ Labeling Kit | 8G |
| PR09 | Refraction-2D™ Labeling Kit | 12G |
| PR10 | Refraction-2D™ Labeling Kit | 18G |
| PR11 | Refraction-2D™ Labeling Kit | 24G |
| PR12 | Refraction-2D™ Labeling Kit | 36G |
| PR13 | Refraction-2D™ Labeling Kit | 72G |
| PR14 | Refraction-2D™ Spot Picking Kit | 3G |

Saturn-2D™ protein labeling kits

| Prod. No. | Description | Kit size |
|-----------|-------------------------|------------------|
| PR31 | Saturn-2D™ Labeling Kit | 4S |
| PR32 | Saturn-2D™ Labeling Kit | 8S |
| PR33 S | Saturn-2D™ Labeling Kit | Preparative + 8S |

Protein labeling kits + analysis software Delta2D

| Prod. No. | Description | Kit size |
|-----------|----------------|-------------|
| PR41 | 2DXPLORER™ Kit | 4 Analyses |
| PR42 | 2DXPLORER™ Kit | 12 Analyses |
| PR43 | 2DXPLORER™ Kit | 24 Analyses |
| PR44 | 2DXPLORER™ Kit | 48 Analyses |
| PR45 | 2DXPLORER™ Kit | 96 Analyses |

Related literature (pdf downloads)

www.dyeagnostics.com/site/products/refraction-2d/manuals

- Refraction-2D™ Product Guide
- Saturn-2D™ Product Guide
- T-Dye Series Product Guide
- Spot Picking Guide
- Imaging Guide
- Research Service Brochure
- Protein Labeling Kits Brochure

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