NH DyeAGNOSTICS

Product Guide

Refraction-2D™ QPLEX Labeling Kit

Product no. PR60, PR61, PR62

NH DyeAGNOSTICS GmbH
Weinbergweg 23
D-06120 Halle

Technical Support
Fon: +49 (0) 345-2799 6413
e-mail: service@dyeagnostics.com
www.dyeagnostics.com

Rev. 01/2018 (1)

FOR RESEARCH USE ONLY

1 Products and content

	RF-2D QPLEX Kit 4G	RF-2D QPLEX Kit 8G	RF-2D QPLEX Kit 12G
Product no.	PR60	PR061	PR62
G-Dye 100	1x 4G	2x 4G	1x 12G
G-Dye 200	1x 4G	2x 4G	1x 12G
G-Dye 300	1x 4G	2x 4G	1x 12G
G-Dye 400	1x 4G	2x 4G	1x 12G
G-Dye Solvent	1x	2x	1x
G-Dye Labeling Stop Solution	1x	2x	1x
G-Dye Low-retention Tubes* & Tipps	1x	1x	1x
Extra G-Dye 100	---	---	1x 3G

The declaration 4G, 8G und 12G indicates the number of large 2D gels that can be performed with the kit.

** Note: Use only suitable rotor for centrifugation.*

2 Storage and stability

Store G-Dyes, G-Dye Solvent and G-Dye Labeling Stop Solution dark at -20°C to -80°C.

Best before: see kit package

Short-term storage (< 2h) of dissolved G-Dyes: +2 to +8°C. Long-term storage of dissolved S-Dyes: -20 to -80°C. Use dissolved G-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 G-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:

- Refraction-2D™ compatible sample buffer (see 7.2)
- 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 blue, green, red and infrared
- Software for data evaluation (e.g. Delta 2D; available at www.dyeagnostics.com)

5 General Information

Developed for modern 2D gel based top down proteomics analyses Refraction-2D™ QPLEX offers direct and sensitive comparison of up to 4 protein samples using only one gel (sample multiplexing). Furthermore, its easy and accurate spot picking feature allows the isolation of candidate proteins without additional gel staining (see Spot picking guide).

Refraction-2D™ QPLEX Labeling Kits are user-friendly by the addition of solvent, stop solution and dye saving pipette tips. The contained high performance fluorescent G-Dyes are extremely photostable and do not require sample preparation at low light conditions. Refraction-2D™ QPLEX gels - after fixation- can be scaned several months later and provide still high quality images.

G-Dye100, G-Dye200, G-Dye300 and G-Dye400 high performance fluorescence dyes are activated as NHS-ester for covalent labeling of lysine residues of proteins. Refraction-2D™ QPLEX labeling protocol ensures that approximately 3% of all proteins are

labeled with one dye molecule per protein and therefore allows quantitative 2D gel analyses.

The different molecular weights of the G-Dyes leads to a reduction of fluorescence interference of the different channels and coupled to this to an increased fluorescence performance. Using appropriate imaging devices, G-Dye labeled protein amounts as low as 0.03 ng can be detected.

Refraction-2D™ QPLEX images can be analyzed by all software suitable for the analyses of 2D gels (www.dyeagnostics.com/site/de/technology/software).

The G-Dyes are compatible to all conventional gel staining methods like silver or Coomassie brilliant blue and do not interfere with subsequent protein identification by mass spec.

For questions please contact us at service@dyeagnostics.com.

6 Overview: Refraction-2D™ QPLEX Labeling

1. Experimental design
2. Solubilisation of proteins in compatible sample buffer
3. Preparation of an internal standard (IS)
4. Preparation of the G-Dye working solution
5. Labeling of protein samples for Refraction-2D™ QPLEX gels
6. Fluorescence imaging

7 Detailed protocol for Refraction-2D™ QPLEX Labeling

7.1 Experimental Design

For comparison of three protein samples (e.g. sample A control vs. sample B 12 h treatment vs. sample C 24 h treatment) use one 2D gel (plus replicates). Analyse three samples and the internal standard (IS) within one 2D gel. The IS represents a mixture of all protein samples of your experiment. This allows easy gel-to-gel-comparison.

Small Refraction-2D™ QPLEX experiments (≤ 12 samples) require technical replicates of the 2D gels. Use biological replicates to discriminate between the natural variance and differences of the protein expression level. Since in general fluorescence dyes differ slightly in their binding preference to each protein dye-swaps should be included to the experiment.

Label for a Refraction-2D™ QPLEX gel (size approx. 22 x 24 cm, total protein load 200 µg) each sample (50 µg) with 1G of G-Dye. 1G = 1 µl G-Dye working solution.

Example of Dye-Swap:

Gel 1:
sample A (50 µg) labeled with G-Dye200 +
sample B (50 µg) labeled with G-Dye300 +
sample C (50 µg) labeled with G-Dye400 +
IS (50 µg) labeled with G-Dye100

Gel 1:
sample A (50 µg) labeled with G-Dye300 +
sample B (50 µg) labeled with G-Dye400 +
sample C (50 µg) labeled with G-Dye200 +
IS (50 µg) labeled with G-Dye100

Gel 1:
sample A (50 µg) labeled with G-Dye400 +
sample B (50 µg) labeled with G-Dye200 +
sample C (50 µg) labeled with G-Dye300 +
IS (50 µg) labeled with G-Dye100

7.2 Solubilisation of proteins in Refraction-2D™ compatible sample buffer

For best labeling results, make sure that your protein is dissolved in a Refraction-2D™ compatible sample buffer (see below). The protein concentration of the sample should be at least 5 µg/ µl [1]. After protein extraction, reassure that the pH of the protein solution is higher than 8.0.

[1] The recommended minimum protein concentration is 2 µg/µl. In this case add 1 µl of G-Dye working solution (1G) and quench labeling with 1/10 volume of labeling stop solution. For protein samples with lower concentration, precipitate the proteins and dissolve your sample in a smaller amount of sample buffer.

Note: For low protein concentrations (< 2 µg/µl) the labeling assay has to be adjusted. Please contact our service team: service@dyeagnostics.com

Refraction-2D™ compatible sample buffer

Do not heat! Store aliquots at -20°C to -80°C lagern.

reagent	concentration	quantity
Tris	30 mM	0,18 g
Urea	7 M	21,00 g
Thiourea	2 M	7,60 g
CHAPS	4% (w/v)	2,00 g

Add deionized water to a total volume of 50 ml; adjust pH to 8.5

7.3 Preparation of an internal 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 G-Dye100 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 5 µg/µl using a Refraction-2D™ compatible sample buffer. Label this mixture using G-Dye100.

Example:

n = 1 gel, protein sample A and B
Mix 25 µg of protein from sample A with 25 µg of protein from sample B, adjust protein concentration to 5 µg/µl and label with 1G
(=1 µl) G-Dye100.

n= 5 gels, protein sample A and B
Mix 125 µg of protein from sample A with 125 µg of protein from sample B, adjust protein concentration to 5 µg/µl and label with 5G (=5 µl) G-Dye100. Distribute the labeled internal standard in equal amounts of 50 µg to the five 2D gels or IPG strips.

7.4 Preparation of the G-Dye Working Solution

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

- Allow vials containing G-Dyes to warm up to ambient temperature (approx. 5 minutes).
- Spin down vials briefly.
- Dissolve G-Dyes in

  4,5 µl of G-Dye solvent for Refraction-2D™ QPLEX **4G, 8G** kit (Product PR60, PR61).

  12,5 µl of G-Dye solvent for Refraction-2D™ QPLEX **12G** kit (Product PR62).

- Vortex and spin down briefly. The G-Dye working solution is now ready for further use.

7.5 Labeling of protein sample for Refraction-2D™ QPLEX analysis

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

- Transfer 50 µg (optimal: ≤ 10 µl; max. 25 µl) of protein (e.g. sample 1) to a fresh G-Dye micro centrifuge tube.

  Note: For low protein concentrations (< 2 µg/µl) the labeling assay has to be adjusted. Please contact our service team: service@dyeagnostics.com

- Add compatible sample buffer to a total volume of 10 µl.
- Vortex and spin down briefly*.
- Add 1 µl G-Dye working solution. Vortex and spin down briefly*.
- Incubate on ice for 30 minutes.
- Quench labeling reaction by adding 1 µl labeling stop solution.
- Vortex and spin down briefly*. Incubate on ice for 10 minutes.
- Protein sample can now be used for further analysis (e.g. IEF).
- optional: check for labeling bei 1D SDS-PAGE (recommended amount of protein: 1 µg per lane).

  ** Note: Use only suitable rotor for centrifugation.*

7.6 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 G-Dye labeled protein gels after finishing SDS-PAGE.
Please find further information on www.dyeagnostics.com/

G-Dye excitation and emission parameter

G-Dye	max. excitation [nm]	max. emisson [nm]
G-Dye100	498	524
G-Dye200	554	575
G-Dye300	648	663
G-Dye400	736	760

8 Post-elektrophoretische Anwendnungen

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.

G-Dye label does not interfere with protein identification by mass spectrometry, enymatic digestions or sequence coverage.

G-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 G-Dye fluorescence signals).