NH DyeAGNOSTICS

Refraction-2D™ QPLEX

Product Guide

Refraction-2D™ QPLEX Labeling Kit

Product no. PR60, PR61, PR62

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Refraction-2D™ QPLEX Labeling Kit

Kit contents

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

- G-Dye100 – high performance fluorescent dye
- G-Dye200 – high performance fluorescent dye
- G-Dye300 – high performance fluorescent dye
- G-Dye400 – high performance fluorescent dye
- G-Dye labeling stop solution
- G-Dye solvent
- G-Dye low retention pipette tips (mass spec compatible)
- G-Dye micro centrifuge tubes (mass spec compatible)

For kit sizes 12G:
- 1 vial containing G-Dye100 sufficient for the labeling of 150 µg of protein for easy and accurate spot picking

Spot picking guide download:
http://www.dyeagnostics.com/site/wp-content/uploads/2011/01/SPG_DetailedGuide_eng_VKC_070512.pdf

Caution

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.

Tested quality and continuous control

In order to ensure a constant quality of your analyses all G-Dyes and Refraction-2D™ QPLEX Protein Labeling Kits underlie a most rigid quality control. Every batch is controlled for sensitivity and labeling efficiency. Only kits that succeeded quality control will be shipped to our customers. All batches get controlled bi-weekly until expiration date.

Refraction-2D™ QPLEX Labeling Kit

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. The Refraction-2D™ QPLEX labeling protocol ensures that approximately 3% of all proteins are labeled with one dye molecule per protein and therefore allows quantitative QPLEX 2D gel analyses.

The different molecular weights of the G-Dyes lead 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™ 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 allow subsequent protein identification by mass spec.

Protocol for Refraction-2D™ QPLEX analysis (general procedure)

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
6. Fluorescence imaging

For further information or questions please contact us at *service@dyeagnostics.com.*

1. Experimental design

For comparison of three protein samples (e.g. sample A *control*, sample B *treatment 12h* vs. sample C *treatment 24h*) use one 2D gel (plus replicates). Analyze 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.

Use biological replicates to discriminate the natural variance from differences of the protein expression level in samples. Since in general fluorescence dyes differ slightly in their binding preference to each protein dye-swaps should be included to the experiment.

Label for one Refraction-2D™ QPLEX gel (size approx. 22 x 24 cm, 4 samples, total protein load 200 µg) each sample (50 µg) with 1G unit of G-Dye. 1G = 1 µl G-Dye working solution.

Dye-Swap (e.g.):

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

Gel 3: 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

2. Solubilisation of proteins in compatible sample buffer

For best labeling results, make sure that your protein is dissolved in a Refraction-2D™ QPLEX 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 1 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.*

Refraction-2D™ compatible sample buffer

Do not heat buffer to dissolve urea. Store in aliquots at -20°C.

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

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 recommend 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™ QPLEX compatible sample buffer. Label this mixture using 1 G (=1 µl) G-Dye100.

Example:
n = 1 gel, protein sample A, B and C
Mix 16.7 µg of protein from sample A with 16.7 of µg protein from sample B and 16.7 µg of protein from sample C, adjust protein concentration to 5 µg/µl and label with 1G (=1 µl) G-Dye100.

n = 5 gels, protein sample A, B and C
Mix 83.3 µg of protein from sample A with 83.3 µg of protein from sample B and 83.3 µg of protein of sample C, 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.

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.

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

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

   12,5 µl of G-Dye solvent for Refraction-2D™ **12G** kit (Product PR62).
4. Vortex and spin down briefly. The G-Dye working solution is now ready for further use.

5. Labeling of protein samples for Refraction-2D™ QPLEX

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

1. Transfer 50 µg (≤ 10 µl) of protein (e.g. sample 1) to a fresh G-Dye micro centrifuge tube.
2. Add compatible sample buffer to a total volume of 10 µl.
3. Vortex and spin down briefly.
4. Add 1 µl G-Dye working solution. Vortex and spin down briefly.
5. Incubate on ice for 30 minutes.
6. Quench labeling reaction by adding 1 µl labeling stop solution.
7. Vortex and spin down briefly. Incubate on ice for 10 minutes.
8. Protein sample can now be used for further analysis (e.g. IEF).

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. Gels stored within low fluorescent glass cassettes (product no PR03 and PR04) can be scanned 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.

Please find further information on www.dyeagnostics.com/site/en/products/refraction-2d/.

G-Dye excitation and emission properties

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

Storage

Store G-Dyes at -20°C to -80°C in the dark. Label proteins within three weeks after solubilization of G-Dyes. Store labeled proteins for up to three months at -80°C.

Best before: see quality label