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Protein labeling with 3Dye differential proteomic reagents

Differential proteomics allows to compare big sets of proteins originating from various biological sources. Usual examples are treated and untreated cells, or differential bacterial strains. Small differences in big number of components can be discovered by differential proteomics.

Lumiprobe 3Dye Kit comprises Cyanine2, Cyanine3, and Cyanine5 dyes which are spectrally distinct, but mobility-matched. Thus, proteins labeled with these dyes co-migrate in gel-electrophoresis. Proteins are labeled with the above dyes via NHS ester chemistry. So, lysines are predominant sites of the labeling.

Best practice of 2D proteomics makes use of internal pooled control sample which is essentially a mixture of both proteomes labeled with one of the dyes. Cyanine2 should be used for it. Other two dyes, Cyanine3, and Cyanine5, are interchangeable for all experiments.

3Dye reagents come pre-measured in 5 nmol, 10 nmol, and 25 nmol packaging. Dyes are lyophilized to prolong shelf life. Each package should be re-constituted with DMF prior to use. DMF quality is essential for the experiments, and product shelf life. DMF comes with the dyes in the kit.

Protein mixture preparation is the most variable, and most demanding part of the assay. It is outside the scope of the protocol because different samples require very different lysis conditions to obtain mixture of proteins suitable for the assay. Essentially, both samples being compared should be lysed in identical conditions to achieve meaningful results. Generally, if experiment requires lysis, CHAPS-based lysis solutions are recommended.

After the preparation of both protein mixtures being compared, the following protocol can be used to achieve labeling.

1. Prepare 1 mM stock solution of each dye by adding 1 uL of DMF (from kit) per 1 nmol of each dye. These solutions are stable for three months at −20°C. To ensure shelf life, desiccate, completely unfreeze the tubes before opening, purge with inert gas when possible before closing
2. Adjust protein mixtures pH to 8.5 by using either amine-free buffer ($NaHCO_3$, acetate), or Tris buffer. In separate vials, prepare three labeling reactions: one untreated, one treated, and one mix of both (pooled internal control). Protein concentration should idealy be 5-10 mg/mL, but as low as 1 mg/mL can be used. Take 50 ug of protein per reaction.
3. Prepare working solutions of dyes by taking aliquots of 1 mM stock solution and diluting with DMF to 0.4 mM.
4. Add 1 uL of working dye solution to reaction mixture: Cyanine3 for untreated, Cyanine5 for treated (or vice versa), and Cyanine2 for pooled internal control. Mix each reaction by pipetting it in and out, and leave for 30 min in the dark.
5. Stop the reactions by adding 1 uL of 10 mM lysine to each solution (it is not included in the kit, but this reagent is readily available)
6. Pool the three samples, and run 2D gel.
7. Analyze with any imager capable of detecting Cyanine2, Cyanine3, and Cyanine5. Protein spots

can be cut, and analyzed by mass-spectrometry.