Protein labeling with differential proteomic reagents

Primetech provides By-2 NHS ester minimal dye, By-3 NHS ester minimal dye, By-5 NHS ester minimal dye 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. By-2 NHS ester minimal dye should be used for it. Other two dyes, By-3 NHS ester minimal dye and By-5 NHS ester minimal dye, are interchangeable for all experiments.

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 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: By-3 NHS ester minimal dye for untreated, By-5 NHS ester minimal dye for treated (or vice versa), and By-2 NHS ester minimal dye 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 By-2 NHS ester minimal dye, By-3 NHS ester minimal dye, By-5 NHS ester minimal dye. Protein spots can be cut, and analyzed by mass-spectrometry.

You may simly e-mail us to order your kit, just specify the quontity.