2-D Protein Extraction Buffer

Efficient cell lysis and protein extraction are key steps for achieving high quality results in downstream applications such as 2-D gel analysis. 2-D Protein Extraction Buffer offers a convenient way to prepare high quality protein lysates. The buffers are modifications of well-studied protein solubilization buffers, designed to produce high spot resolution for 2-D gel analysis. Six buffers are available, supplied as a dry powder which is rehydrated with the provided DILUENT. The optimal buffer will depend on the nature of your sample, and 2-D Protein Extraction Buffer Trial Kit allows you to evaluate each extraction buffer to find the most suitable buffer for your sample (Fig 1). 2-D Protein Extraction Buffer can also be used prior to 1-D electrophoresis, or for rehydrating IPG strips prior to 2-D electrophoresis.

Key benefits include:

- Ready to mix buffers simplify protein extraction preparation of cells and tissues
- 2-D Protein Extraction Buffer Trial Kit facilitates easy optimization of total protein extraction
- Efficient and reproducible protein extraction with high yield
- Comprehensive selection of six different buffers covering the wide range of protein diversity
- Complete declaration of buffer components, providing transparency about what you are putting into your sample

Fig 1. 2-D Protein Extraction Buffer offers convenient and efficient protein extraction. 2-D Protein Extraction Buffer Trial Kit includes all six extraction buffers, allowing you to determine the most suitable extraction buffer for your sample. 2-D Protein Extraction Buffer packs include dry buffer component and DILUENT.

Description

Six protein extraction buffers are available, 2-D Protein Extraction Buffer-I to -VI, which are based on urea, thiourea, CHAPS and additional solubilizing agents as shown in Table 1. Because the buffers are provided in a dry powder formulation, problems associated with carbamylation are avoided. Carbamylation, which can occur in solutions containing urea, may alter protein charge and produce spot artifacts on 2-D spot maps. With 2-D Extraction Buffers, buffer can be freshly made by weighing out the necessary amount and resuspending it in the included DILUENT. Each package contains enough reagents necessary to prepare 50 ml buffer, and the 2-D Protein Extraction Buffer Trial Kit includes reagents for preparing 10 ml of each of the six buffers.

Table 1. Composition of 2-D Protein Extraction Buffer and 2-D Protein Extraction Buffer Trial Kit

2-D Protein Extraction Buffer	Composition
2-D Protein Extraction Buffer-I with DILUENT-I, 50ml	Urea (< 10 M) and NP-40* (< 10%)
2-D Protein Extraction Buffer-II with DILUENT-II, 50ml	Urea (< 10 M) and CHAPS† (< 10%)
2-D Protein Extraction Buffer-III with DILUENT-III, 50ml	Urea (< 8 M), Thiourea (< 5 M), CHAPS (< 5%), and ASB-16‡ (< 5%)
2-D Protein Extraction Buffer-IV with DILUENT-III, 50ml	Urea (< 8 M), Thiourea (< 5 M), CHAPS (< 5%), and SB 3-10§ (< 5%)
2-D Protein Extraction Buffer-V with DILUENT-II, 50ml	Urea (< 8 M), Thiourea (< 5 M), and CHAPS (< 10%)
2-D Protein Extraction Buffer-VI with DILUENT-III, 50ml	Urea (< 8 M), Thiourea (< 5 M), CHAPS (< 5%), and NDSB-201¶ (< 4%)
2-D Protein Extraction Buffer Trial Kit, 6 x 10 ml	2-D Protein Extraction Buffer-I, -II, -III, -IV, -V and –VI, including DILUENT

*Nonylphenyl polyethylene glycol †3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate ‡Amidosulfobetaine-16 §n-Decyl-N,N-dimethyl-3-ammonio-1-propanesulfonate ¶3-(1-Pyridino)-1-propane sulfonate

Applications

Differential extraction of rat liver proteins

This study presents a comparison between different protein extraction buffers to investigate the extraction capabilities and differences between the buffers. Rat liver proteins were extracted using 2-D Protein Extraction Buffer-I to -VI, as well as a standard 2-D lysis reference buffer (8 M urea, 2 M thiourea, and 4% CHAPS). After protein extraction, the samples were analyzed using 2-D Fluorescence Difference Gel Electrophoresis (DIGE).

Before use, the different extraction buffers were resuspended in the provided DILUENT according to the instructions. In addition, the reference buffer and extraction buffers were supplemented with 30 mM Tris-HCl (pH 8) and Protease Inhibitor Mix (1X). Figure 2 shows the workflow, including some of the key products that were utilized at each step. For homogenization, the Sample Grinding Kit was used. In this step, 0.1 ml reference buffer or 2-D Protein Extraction Buffer was mixed with tissue samples (35 to 50 mg), in triplicate, in tubes containing grinding resin. After grinding for 1 min at room temperature, 0.3 ml of the same extraction buffer was added and the samples were vortexed. The lysate was then cleared by centrifugation (15 000×g, 20ºC, 20 min), and the protein concentration in the cleared supernatant was quantitated using the 2-D Quant Kit. Finally, the pH of the lysates was adjusted to pH 8 by adding 0.5 M NaOH.

The average yield for the triplicate samples was similar for the six different extraction buffers, ranging from 0.13–0.16 mg protein/mg tissue. These yields were also comparable to the reference buffer which resulted in 0.16 mg protein/mg tissue. However, in general, 2-D Protein Extraction Buffer-III, -IV, -V, and -VI are known to give stronger solubilization effects and may therefore be more appropriate for harder tissues such as muscle or membrane protein-rich cells.

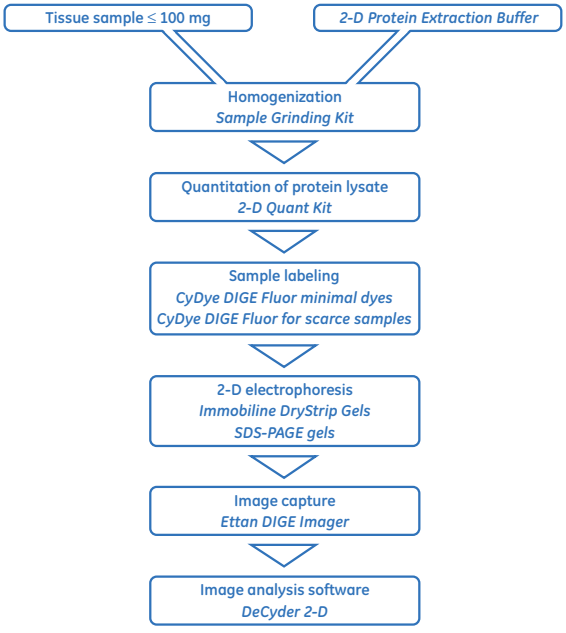

Fig 2. Workflow using 2-D DIGE to investigate differences in extraction capabilities of 2-D Protein Extraction Buffer-I to -VI.

Compatibility with CyDye

Before performing 2-D DIGE, a 1-D study was carried out to test the compatibility of 2-D Protein Extraction Buffer with CyDye™ DIGE Fluors. In this study, the protein lysates, extracted using the reference buffer or the different extraction buffers, were labeled with Cy™5 minimal dye or Cy5 saturation dyes for scarce samples according to the instruction manual. In these labeling reactions, 50 µg of protein was labeled with the minimal dye and 5 µg protein was labeled using the CyDye DIGE Fluor for scarce samples. After labeling, the samples were analyzed by SDS-PAGE, scanned using the Ettan™ DIGE Imager, and fluorescence intensity was assessed. Figure 3 shows the protein samples labeled with the minimal dye, and this figure demonstrates that CyDye DIGE Fluor minimal dyes are compatible with the majority of the extraction buffers, although the labeling intensity was slightly reduced in samples extracted by 2-D Protein Extraction Buffer–I. For the saturation dyes, a reduced labeling efficiency was observed for samples extracted using 2-D Protein Extraction Buffer-III and –IV, and these buffers are therefore not recommended when saturation dyes will be used for labeling.

2-D DIGE analysis

2-D DIGE was performed on the protein lysates extracted with reference buffer or 2-D Protein Extraction Buffer-II to -VI so that the resulting 2-D spot maps could be compared. 2-D Protein Extraction Buffer-I was not tested, since this extraction buffer is not recommended when using CyDye minimal dyes for labeling. After protein extraction, the samples were labeled with Cy3 and Cy5 minimal dyes, and the pooled standard was labeled with Cy2 minimal dye, according to the instructions. For first dimension

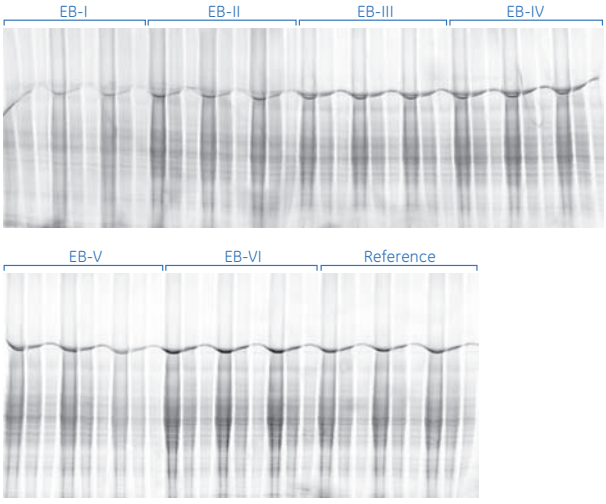

Fig 3. Rat liver samples were extracted using either the reference buffer or 2-D Protein Extraction Buffer-I to -VI. Protein extracts were subsequently labeled with a CyDye minimal dye, and run on Excel Gel 8–18. In each set of three lanes, 20, 10 or 5 µl of sample was loaded. Most of the extraction buffers were compatible with minimal dyes, and gave a high labeling intensity compared to the reference, although Extraction Buffer (EB)-I gave a reduced labeling intensity and is therefore not recommended when using CyDye minimal dyes.

electrophoresis, samples were run on a Multiphor™ II instrument using 24 cm Immobiline™ DryStrip Gels (pH 3–11 NL). The strips were then loaded onto a second dimension polyacryamide gel (12.5%). Finally, the gels were scanned using the Ettan DIGE Imager and analyzed with DeCyder™ 2-D Differential Analysis Software.

The results of the study showed that some spots were differentially extracted by the buffers, which may be of importance in maximizing yield for preparative gels and peptide mass fingerprint identification. This also highlights that the most appropriate extraction buffer will not only depend on the nature of your sample, but also on the number of protein species and which protein species that you want to detect in your 2-D analysis. Figure 4 shows the spot map from a sample extracted using 2-D Protein Extraction Buffer-II overlaid on the spot map using Extraction Buffer–VI for extraction. Several protein spots were differentially extracted by these buffers, displayed in the figure as spots shifting towards red (extracted to a higher degree with Extraction Buffer-II) and spots shifting towards green (higher abundance after extraction with Extraction Buffer-VI). The most apparent difference between the extraction buffers was between 2-D Protein Extraction Buffer-II and the rest of the buffers, probably because thiourea is included in Extraction Buffer-III to -VI. Figure 5 shows a DeCyder software analysis comparing the abundance of a single protein spot after extraction with the different extraction buffers. Figure 5A shows a protein spot that was preferentially extracted by 2-D Protein Extraction Buffer-II. Note that the thiourea-based reference buffer gave an extraction efficiency that was similar to Extraction Buffer-III, -IV, -V, and -VI. Figure 5B shows a different protein spot where 2-D Protein Extraction Buffer-III to -VI showed slightly different extraction capabilities, with Extraction Buffer-VI giving the highest abundance. However, in general, the thiourea-based extraction buffers produced only subtle differences in the resulting 2-D spot maps.

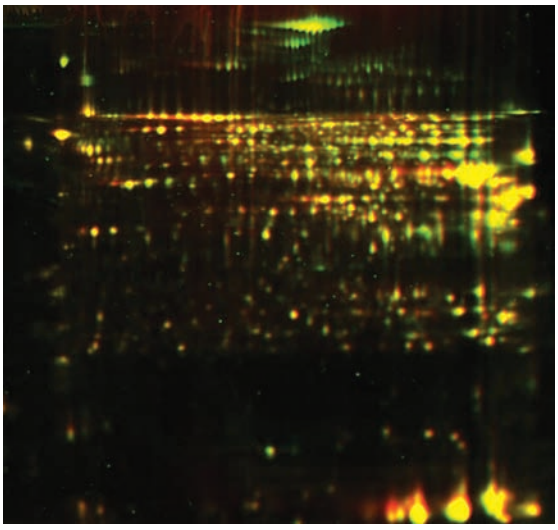

Fig 4. 2-D DIGE gel image of a protein sample extracted using Extraction Buffer-II overlaid on a sample extracted by Extraction Buffer–VI. Protein spots shifting towards red were extracted to a higher degree with Extraction Buffer-II, while spots shifting towards green had a higher abundance after extraction with Extraction Buffer-VI. The most distinct difference between the six extraction buffers was found between Extraction Buffer-II and the rest of the buffers.

2-D Protein Extraction Buffers may also be used sequentially in order to utilize the different extraction powers of the buffers and maximize the amount of protein extracted from a sample. For example, protein lysate is first made using a urea-based buffer such as Extraction Buffer-I or -II. Sequential

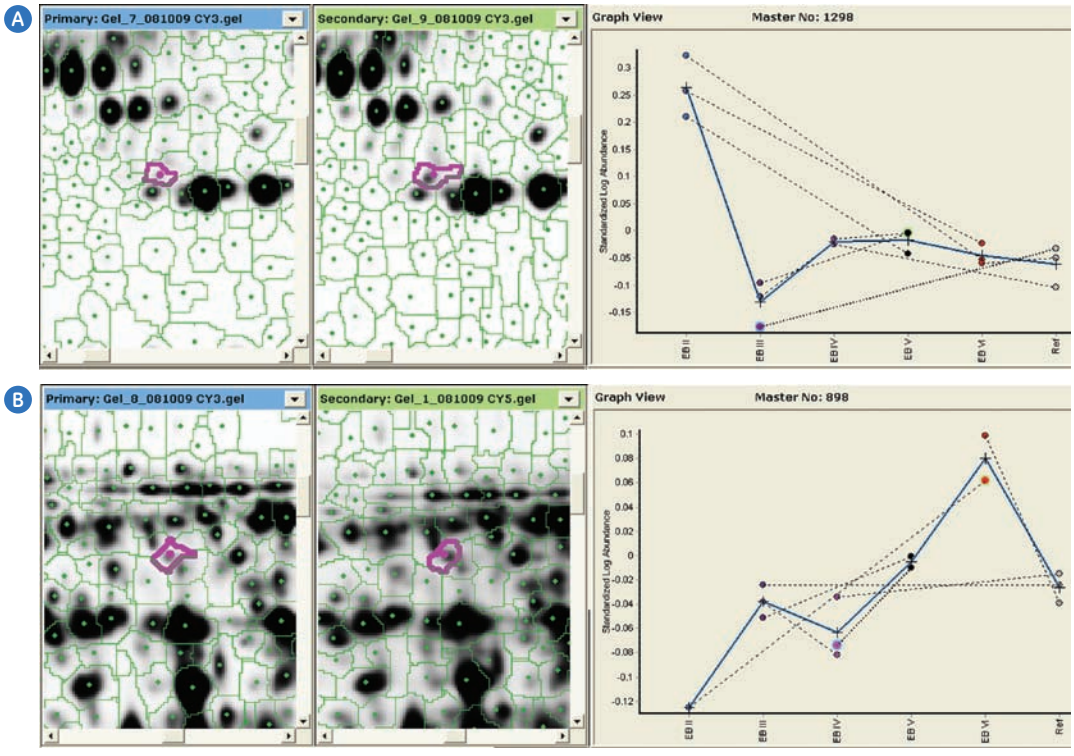

Fig 5. DeCyder software analysis showing protein abundance of the same protein spot (shown to the left on two different gels) extracted using different extraction buffers. A) The protein spot was extracted to a higher degree with Extraction Buffer-II. B) Another example showing a protein spot that gave the highest abundance with Extraction Buffer-VI. Extraction capability depended on the protein spot, but in general, the thiourea-based extraction buffers—Extraction Buffer-III to -VI and the reference buffer—resulted in similar 2-D spot maps.

extractions are then carried out on the lysate, for example, by performing a second extraction with Extraction Buffer-V and a third extraction with either Extraction Buffer –III, -IV, or -VI. After each extraction step, new protein spots can be extracted and identified.

For more information regarding sample preparation solutions, refer to Appendix I in the handbook "2-D Electrophoresis: Principles and Methods." In Appendix I, Section A, the chemical components are the same components found in 2-D Protein Extraction Buffer-II (except for IPG Buffer and DTT). In Section B, the chemical components are the same as the components found in 2-D Protein Extraction Buffer-V (except for IPG Buffer and DTT).

Other applications

2-D Protein Extraction Buffer can also be used prior to 2-D electrophoresis for the reswelling of Immobiline DryStrip Gels (IPG strips) in acidic to neutral pH intervals as an alternative to DeStreak Rehydration Solution. To simplify reswelling, IPGbox can be used to rehydrate up to twelve IPG strips in individual slots in a minimum volume of solution.

Ordering information

Product	Quantity	Code no
2-D Protein Extraction Buffer-I	For 50 ml	28-9435-23
2-D Protein Extraction Buffer-II	For 50 ml	28-9435-24
2-D Protein Extraction Buffer-III	For 50 ml	28-9435-25
2-D Protein Extraction Buffer-IV	For 50 ml	28-9435-26
2-D Protein Extraction Buffer-V	For 50 ml	28-9435-27
2-D Protein Extraction Buffer-VI	For 50 ml	28-9435-28
2-D Protein Extraction Buffer Trial Kit	For 6 x 10 ml	28-9435-22

Related products

Product	Quantity	Code no
Sample preparation		
Yeast Protein Extraction Buffer Kit		28-9440-45
Mammalian Protein Extraction Buffer		28-9412-79
Sample Grinding Kit	50 samples	80-6483-37
Albumin and IgG Removal Kit	10 samples	RPN6300
2-D Quant Kit	500 assays	80-6483-56
2-D Fractionation Kit	10 samples	80-6501-04
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23
2-D Clean-Up Kit	50 samples	80-6484-51

Related Literature

2-D Electrophoresis: Principles and Methods	80-6429-60
Ettan DIGE System User Manual	18-1173-17

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