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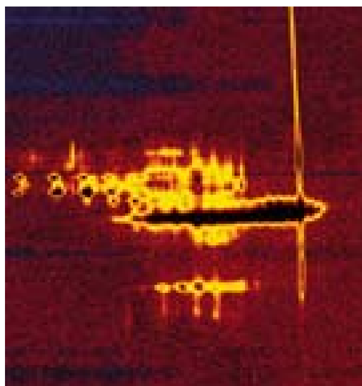
bio Analytix
Advances in Analytical Chemistry

SIGMA-ALDRICH

Technology and General Advantages



Principle

Isoelectric focusing (IEF) is an electrophoretic separation based on the isoelectric points of proteins. The pI is the point at which the protein has an overall net charge of zero. Differences of only a few hundredths of a pH-unit in isoelectric points are sufficient to resolve proteins from each other. IEF is used as an alternative electrophoresis format complementing the widely used SDS-PAGE electrophoresis, which is based on size of proteins. The combination of both principles is applied in 2D-electrophoresis as outlined below (see page 2). This technique has become crucial for the development of Proteomics. In many applications, closely related proteins have to be separated. Examples are the differentiation of protein isoforms or enantiomeres. Such problems have been successfully solved by isoelectric focusing (IEF) [1]

In contrast to other electrophoretic techniques, pH is not kept constant throughout the whole system. Instead, the sample components migrate electrophoretically in a stationary pH-gradient. Proteins will migrate until they reach the pH-point in the gradient at which the charge of the protein equals zero (pH = pI) The protein is said to focus at this point. This focusing also results in a concentration of individual proteins (Figure 1).

Advantages

Optimal resolution in other electrophoretic systems requires application of sample as a narrow zone. In IEF this is not so crucial, even large sample volumes do not lower resolution. Sample concentration upon focusing also results in lower detection limits compared with other electrophoresis techniques.

Another advantage of this technology is that separation in IEF does not require denaturation of proteins, thus any kind of subsequent investigations, such as activity staining (e.g. to find separated enzymes) or antibody detection, is not hindered.

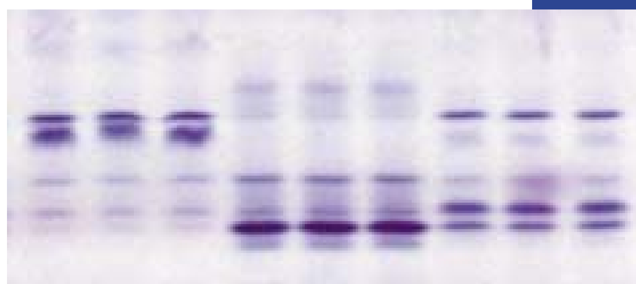


Figure 1:
IEF of maize seeds.
From left to right side: female, male, and F1 generation seed.

Compared to alternative methods (e.g. PCR based), IEF offers the following advantages

- efficient
- expressive
- economic (no sophisticated equipment required)
- easy (clear, one-dimensional separation principle)
- fast
- sensitive

Isoelectric Focusing

Fluka

The Role of Carrier Ampholytes

IEF requires a stationary and stable pH gradient oriented so that the pH increases towards the cathode. Such a gradient can be formed by using a mixture of specially designed amphoteric (both acidic and basic) substances, which are typically referred to as carrier ampholytes. Like proteins, these substances possess net charges that vary with pH and have different pIs. If carrier ampholytes are subjected to an electrical field, they will arrange themselves in such way that the ones with lowest pIs will end up at the anode and those with the highest pIs at the cathode. Then the net charge of all the ampholytes will be zero and the gradient stationary. Their diffusion is controlled by the electric field. This kind of focusing of ampholytes and their buffering properties, results in a stable pH gradient for separation of biomolecules.

Ampholytes can be bound to acrylamide. By using that type of ampholytes (see Table 1 and 2) for polymerization of gel, an immobilized pI-gradient can be formed. Immobilized pI-gradients overcome some otherwise common problems in IEF. They do not show cathodic drift, thus providing higher reproducibility. They also allow application of large samples as the pI-gradient is stable and won't break down. Time savings and convenience also support their use. On the other hand, these immobilized ampholytes will not be able to coelute with proteins in the second dimension of 2D-electrophoresis, therefore allowing better resolution when compared to free ampholytes.

Special Edition!
Protein Analysis and
Fluorescent Probes

IEF in Food and Seed Testing and Diagnostic Applications

Even pure preparations of proteins provide different bands in IEF. This is due to micro-heterogeneity of charges, caused by different post-translational modifications, e.g. different grades of glycosylation or desamination. This often provides clearly distinct species-specific patterns. This is a great advantage, especially for food analysis, seed testing or certain diagnostic applications (Figure 2).

That and the practical advantages mentioned earlier have made IEF the method of choice for applications like

- general characterization of proteins by pI purity determination of proteins
- control of foodstuff, e.g. discrimination of animal species (meat)
- discrimination of caseins
- species characterization of plant material, e.g. wheat
- routine clinical analyses, e.g. liquor, α-amylase

IEF in 2-D-Electrophoresis

Isoelectric focusing has gained importance as the first dimension of 2D-electrophoresis. For these purposes the first dimension is typically applied on strips carrying immobilized pH gradients. These are prepared prior to electrophoresis by pouring the pH gradient with the acrylamide-derivatized buffer that becomes polymerized in the gel (see following section)

Strips may be purchased in ready-to-use format or set up individually using Fluka's well established acrylamido buffers (see Table 3). Sigma's new ProteoGel IPG strips are available in three lengths to accommodate various gel sizes and six pH-ranges to allow optimal separation (Figure 3).

Ready-made strips offer several advantages:

- narrow and wide range strips, with overlap options, allow optimal resolution of most protein samples
- control in manufacturing ensures reproducible performance
- IPG strips reduce both preparation time and reagent waste

Rules for selection of IPG strips include:

- use shorter strips when interested in only the most abundant proteins
- longer strips provide better resolution and increased loading capacity
- for total protein disruption, use strips in the 3–10 pH-range
- use strips in a narrow pH-range to closely study proteins in your range of interest
- combine strips in pH-ranges 4–7 and 6–11 (or 5–8 and 8–11) to obtain greater detail.

Ampholytes play an important role in sample preparation. Typically 0.5–2% ampholyte is added to solubilization buffers to support solubilization, to scavenge cyanate ions and to improve precipitation of nucleic acids during centrifugation.

Figure 2:
Different ewes and goats milk cheeses (1,2,3,4) and European standard cheeses (RF 0%, RF 1%) separated by IEF and visualized by silver staining. Run on a ready-to-use gel (CleanGel Ultra, ETC GmbH) rehydrated in 3% high resolution ampholyte (5–7) and 7 M urea. Courtesy of Dr. Hanspeter Schickle.

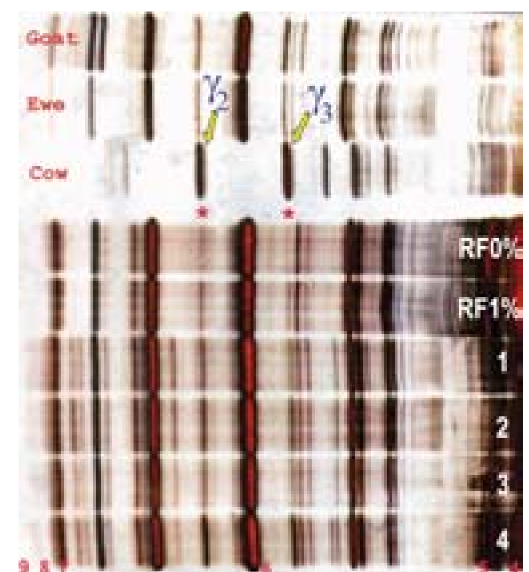
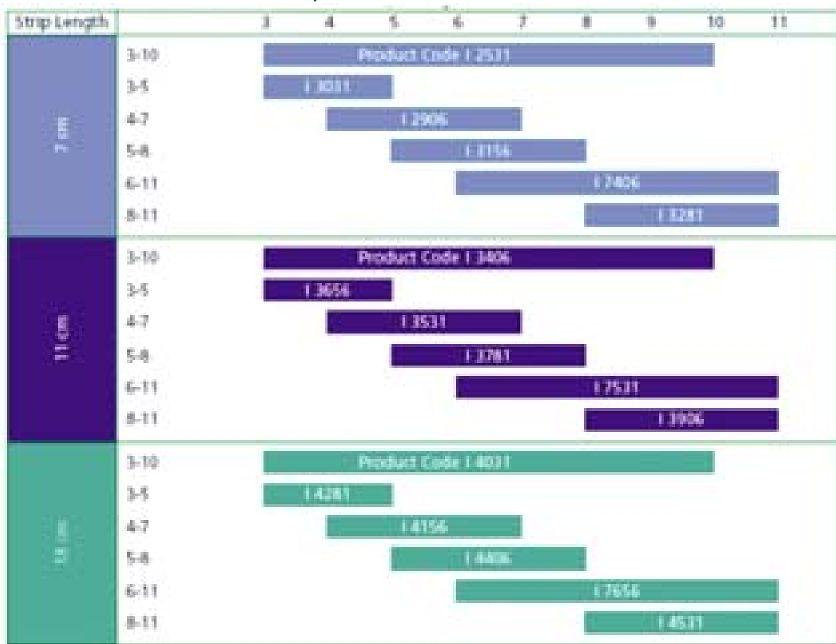


Figure 3:
IPG strips from Sigma-Aldrich



Free ampholytes are also used for rehydration of dry strips before use as shown in Figure 4. There are several protocols for rehydration solutions. A widely used formulation consists of 0.2% ampholyte, 8 M urea, 0.5% CHAPS, and 15 mM DTT. There are other formulations around and concentrations may be higher, e.g. 0.5% ampholyte, 9.5 M urea, up to 2% non-ionic detergent and 1% DTT. In case of hydrophobic proteins 7 M urea and 2 M thiourea have been used instead of only urea. The pH-range of ampholyte should fit the range of the chosen strip. Within our broad range of formulations you will find all the ones to match the strips of your choice. Also our ampholytes are significantly lower priced than those offered as «IPG buffers»!
The rehydration buffer can also be used for preparation of sample. For that purpose 0.5 ml of cell suspension may be mixed with 200–500 ml of rehydration buffer.

Figure 4:
2-D electrophoresis of cytoplasmic and membrane associated proteins from E.coli. Strips were rehydrated in 8 M urea, 2% CHAPS, 18 mM DTE, 0.5% Ampholyte 3–10. Sample was deluted with rehydration buffer before electrophoresis. Staining was performed using ruthenium II tris (batophenanthroline disulfonate). Courtesy of Andreas Lamanda, University Bern

Gel Matrices and Preparation of IEF Gels

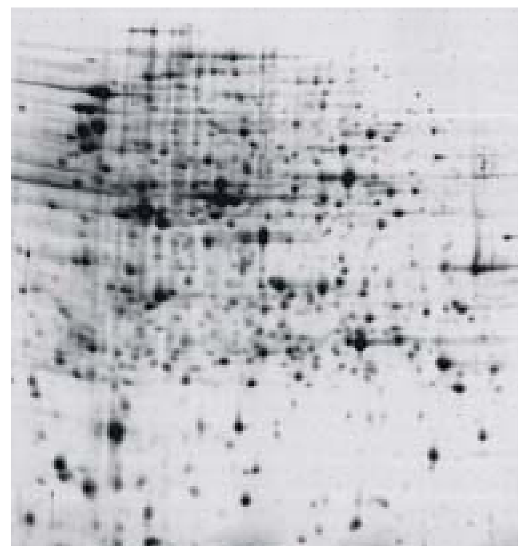
Preparation of polyacrylamide gels for analytical purposes can be achieved using the free carrier ampholytes. For that purpose a final concentration of 2% ampholyte is added to the gel cocktail (solution of acrylamide and bisacrylamide) and handled further as with other types of polyacrylamide gels.
Immobilized pH-gradient gels can be prepared prior to electrophoresis by pouring the pH-gradient with the acrylamide-derivatized buffer that becomes polymerised in the gel. These gels may be set up individually using Fluka's well-established acrylamido buffers (see Table 2). In general, two solutions have to be prepared, one equivalent to the basic end, and the other to the acidic end of the pH-range. As an example for a pI 6–10 gel the following solutions may be used (according to Angelika Görg, for detailed information please see http://www.weihenstephan.de/blm/deg)

	Solution A:	Solution B:
Carrier ampholyte 3.6	941 µl	100 µl
Carrier ampholyte 6.2	273 µl	333 µl
Carrier ampholyte 7.0	243 µl	361 µl
Carrier ampholyte 8.5	260 µl	239 µl
Carrier ampholyte 9.3	282 µl	326 µl
Acrylamide/Bis	2.0 ml	2.0 ml
Water	8.1 ml	11.7 ml
Added in second step:		
TEMED	10 µl	10 µl
Persulfate	15 µl	15 µl

Gels are poured from fresh solutions as for other acrylamide gels.

Polyacrylamide is the most popular matrix for IEF gels, but agarose might still be considered as an alternative, which

- provides larger pore sizes
- is non-toxic and non-mutagenic
- fully compatible with subsequent immunofixation
- allows quick staining and destaining



Agarose of 0.5% to 1.25% may be used. As endoosmotic flow can result in problems, a special quality grade for IEF should be used (e.g. Cat. No. 05056). The preparation is quite easy: after boiling the agarose solution is equilibrated at 56–58°C. Carrier ampholytes are added to a final concentration of 2.5%. The gel solution can then be poured directly on a Gel Bond horizontal sheet or into a vertical cassette as used for polyacrylamide (especially if gel thickness should be below 1 mm)

Our broad range of well-established carrier ampholytes provides you the convenience of various formulations covering the range from pH 2.5 to 10.5 in fractions of different width. Fluka's extensive experience in production of carrier ampholytes enables the reliable high quality of these well-established ampholytes (see Table 1 for products).

High Resolution Ampholytes

A new series of carrier ampholytes (see Table 2) is based on a specific method to produce mixtures of numerous polyamino-polycarboxylic acids. These new carrier ampholytes have been developed for use of isoelectric focusing in quality control applications, especially in food and seed analysis or clinical applications (see Figure 5).

Advantages:

- high buffer capacity: compared to established ampholytes they offer buffering capacities 1.5 - 2.5 times higher (up to pH 8, equal above pH 8)
- good even conductivity
- linear and reproducible pH-gradient
- high resolution

Reviews and Books

[1] P. Glukhovskiy, G. Vigh, Analytical- and Preparative-Scale Isoelectric Focusing Separation of Enantiomers, Anal. Chem., 71 (17), 1999, 3814–3820

[2] Corthals, G.L. et. al., in Rabilloud, T. (ed.): Proteome Research: Two-Dimensional Electrophoresis and Identification Methods, Springer, Berlin Heidelberg New York, 2000, pp. 197–231.

[3] Link, A. (Ed.), Methods in Molecular Biology, 112: 2-D Proteome Analysis Protocols, Humana Press, Totowa, NJ, USA 1999

[4] P.G. Righetti, Isoelectric Focusing: Theory, Methodology and Applications, in Laboratory Techniques in Biochemistry and Molecular Biology, Work, T.S. and Burdon, R.H., eds., 11th printing, 1989

Table 1

Broad and narrow range ampholytes

Cat. No.	Product
10036	Ampholyte pH 3–4.5
10037	Ampholyte pH 3.5–5.0
11923	Ampholyte pH 3.0–6.0
10043	Ampholyte pH 3–10
17099	Ampholyte pH 4.0.–5.0
10038	Ampholyte pH 4.0–6.0
04638	Ampholyte pH 4.0–7.0
17101	Ampholyte pH 4.0–9.0
10048	Ampholyte pH 5.0–7.0
17103	Ampholyte pH 6.6–7.6
10051	Ampholyte pH 6.0–8.0
10053	Ampholyte pH 8.0–9.5
10054	Ampholyte pH 9.0–10.5
10052	Ampholyte pH 7.0–9.0

Table 2

New high resolution ampholytes

Cat. No.	Product
41965	Ampholyte high resolution pH 2–4
79858	Ampholyte high resolution pH 3–5
51304	Ampholyte high resolution pH 3–6
39878	Ampholyte high resolution pH 3–10
68653	Ampholyte high resolution pH 4–6
15752	Ampholyte high resolution pH 5–7
89096	Ampholyte high resolution pH 5–8
89191	Ampholyte high resolution pH 6–8
95632	Ampholyte high resolution pH 7–9
08689	Ampholyte high resolution pH 8–10

Table 3

Acrylamido buffers for preparation of IEF strips

Cat. No.	Product
01712	Acrylamido buffer pK 1, 0.2 M in water
01714	Acrylamido buffer pK 3.1, 0.2 M in water
01713	Acrylamido buffer pK 3.1
01716	Acrylamido buffer pK 3.6, 0.2 M in water
01715	Acrylamido buffer pK 3.6
01718	Acrylamido buffer pK 4.6, 0.2 M in water
01717	Acrylamido buffer pK 4.6
01721	Acrylamido buffer pK 6.2, 0.2 M in n-propanol
01719	Acrylamido buffer pK 6.2
01723	Acrylamido buffer pK 6.6, 0.2 M in n-propanol
01722	Acrylamido buffer pK 6.6
01726	Acrylamido buffer pK 6.85, 0.2 M in n-propanol
01724	Acrylamido buffer pK 6.85
01729	Acrylamido buffer pK 7.0, 0.2 M in n-propanol
01727	Acrylamido buffer pK 7.0
01732	Acrylamido buffer pK 7.4, 0.2 M in n-propanol
01731	Acrylamido buffer pK 7.4
01736	Acrylamido buffer pK 8.5, 0.2 M in n-propanol
01735	Acrylamido buffer pK 8.5
01738	Acrylamido buffer pK 9.3, 0.2 M in n-propanol
01741	Acrylamido buffer pK 10.3, 0.2 M in n-propanol
01739	Acrylamido buffer pK 10.3
01743	Acrylamido buffer pK >12.0, 0.2 M in water

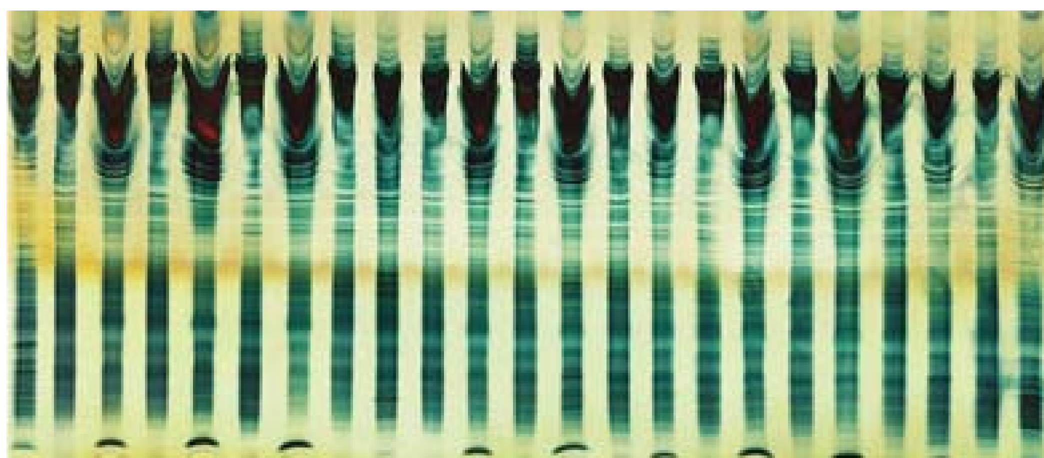


Figure 5: Serum and cerebrospinal fluid samples, one beside each other, run on a ready-to-use gel (Focus-Gel, ETC GmbH) rehydrated in 3% high resolution ampholyte, pH 3–10. Courtesy of Dr. Hanspeter Schickle.

Other reagents for IEF

Table 4

Cat. No.	Product	Description
01698	Acrylamide	
01697	Acrylamide	Solution 40 %
87689	N, N'-Methylene-Bis-Acrylamide	
01705	Acrylamide : Bisacrylamide 19:1	Powdered blend
01693	Acrylamide : Bisacrylamide 19:1	Solution 40%
01704	Acrylamide : Bisacrylamide 24:1	Powdered blend
01706	Acrylamide : Bisacrylamide 24:1	Solution 30%
01702	Acrylamide : Bisacrylamide 29:1	Powdered blend
01708	Acrylamide : Bisacrylamide 29:1	Solution 40%
01703	Acrylamide : Bisacrylamide 32.5:1	Powdered blend
09914	Ammonium persulfate	Starter for polymerization reaction
32664	N,N'-Diallyl L-tartardiamide	Suitable for increase of pore sizes in IEF gels, which makes recovery of proteins from gel easier
51458	Urea	Most common chaotropic reagent used for solubilization and denaturation of proteins before denaturing IEF
51458	Urea	Most common chaotropic reagent used for solubilization and denaturation of proteins before denaturing IEF
88810	Thiourea	Common chaotropic solubilization and denaturation reagent for proteins, esp. for hydrophobic proteins.
42238	Tributylphosphine	Uncharged reducing agent for cystine residues. Reduction before IEF increases resolution in 2D
57670	Iodoacetamide	Alkylation reagent. Complete alkylation prior to IEF improves resolution.
74385	Nonidet® P 40 Substitute	Non-ionic detergent usually used with denaturing IEF, especially in IPG gels
26680	CHAPS	Non-denaturing, zwitterionic detergent suitable for protein solubilization for IEF
75083	Octyl ß-D-glucopyranoside	Non-ionic detergent widely used for IEF, has been shown to be superior to Triton X-100 for IEF
53327	ASB 14;	Highly effective detergent for IEF Amidosulfobetaine-14
40998	Zwittergent® 3–8; 3–(N,N-Dimethyloctyl-ammonio) propanesulfonate inner salt	Zwitterionic sulfobetaine detergent used for solubilization of membrane proteins, CMC 330 mM
30694	Zwittergent® 3–10; 3–(Decyldimethyl-ammonio) propanesulfonate inner salt	Zwitterionic sulfobetaine detergent used for solu-bilization of membrane proteins, CMC 25–40 mM
40232	Zwittergent® 3–12; 3–(Dodecyldimethyl-ammonio) propanesulfonate	Zwitterionic sulfobetaine detergent used for solu- bilization of membrane proteins, CMC 2–4 mM
40772	Zwittergent® 3–14; 3–(N,N-Dimethylmyristyl-ammonio)propanesulfonate	Zwitterionic sulfobetaine detergent used for solubilization of membrane proteins, CMC 0.1–0.4 mM
03550	Ethylendiamine	Cathode buffer component in denatured IEF at high voltages for focusing ultra-thin gels
79617	Phosphoric acid	Suitable for preparation of stock solutions for anode solutions used in IEF
72079	Sodium hydroxide solution 100 mM	Suitable as cathode solution for IEF
G1041	EZBlue™ Gel Staining	Convenient, safe and extremely sensitive colloidal Brilliant Blue G 250 Reagent stain. Colloidal stains are applicable for IEF gels also.
44715	Fast Green FCF	This stain does not interfere with ampholytes and thus well suited for IEF. It provides a wider linear range of detection than Brilliant Blue R.
27816	Brilliant Blue R	Brilliant Blue R is the basis of several direct methods for staining IEF.
27817	Acid violet 17	Rapid staining of proteins in IEF without removal of ampholytes.
47614	Fluoram®	Excellent fluorescent label for pre electrophoresis staining, reacts readily with primary amino groups of peptides to form highly fluorescent compounds.

Sigma-Aldrich – Your Source for Gel Electrophoresis Equipment

Good performance of IEF applications is not only a matter of reagents, but also requires dedicated instruments. Sigma-Aldrich offers a wide range of electrophoresis equipment: electrophoresis units and power supplies, blotting units, incubation dishes, transilluminators and other equipment.

Figure 6 UniPhor horizontal electrophoresis unit

High Performance Horizontal Electrophoresis Units

UniPhor units offer superior performance by innovative Synthetic Carbon Electrodes (SCE) and unique laminar flow «HomoTherm CC» ceran ceramic thermoplates (Figure 6).
In protein analysis these units are well suited for denaturing SDS-PAGE and non denaturing PAA-Gel electrophoresis, IEF in ultrathin PAA-Gel systems and IEF in agarose ampholyte gels, as well as preparative non-denaturating PAGE.

Figure 7 Dual plate electrophoresis unit for 20 x 20 cm gels

These units offer a range of advantages:

- new special electrodes made from synthetic carbon, which are highly resistant to corrosion by oxidation, detergents and pH.
- high-performance «HomoTherm CC» ceran ceramic laminar flow thermoplate (maximum pressure: 1 bar = 14 psi) for maximum gel sizes of approx. 280 x 165 mm (L-Type) and 290 x 200 mm (XL-Type), responsible, for homogeneous thermostating and even heat dissipation, for ultra fast, heavy duty extra-high tension applications.

Vertical Electrophoresis Units

Dual Plate Unit for 10 cm x 10 cm Gels, Cat. No. Z33,957-1
Dual Plate Unit for 20 cm x 20 cm Gels, Cat. No. Z33,985-7
These vertical units can run one or two user-cast gels, or commercially-available pre-cast gels (Figure 7).
They use large buffer volumes in the bottom tank to absorb heat and improve results. The running buffer can be pre-chilled prior to electrophoresis or an additional cooling block connected to a circulating water bath may be used. Gels can be cast directly on the gel-running module using the special casting base. A guaranteed leak-proof seal is obtained using simple clamping bars and screws.
For an overview of our range of equipment, and for details regarding our electrophoresis units, please order your copy of the new Sigma-Aldrich equipment catalog or contact our technical service department.

Isoelectric Focusing in Capillary Electrophoresis

Gel electrophoresis, the common technology for IEF, minimizes convection and intoduces an additional gel-sieving effect to separate proteins by size. However, it has several disadvantages:

- long analysis times
- lack of resolution
- difficulties in detection
- difficult to automate

Capillary electrophoresis is an automatible high-resolution approach to electrophoresis. Separation is carried out in fused-silica capillaries of just 25–75 µm internal diameter. The electrophoresis takes place in free solution and convection currents are controlled by the capillar. After focusing is complete the solutes are pumped out of the capillar. For capillar IEF UV absorption is the most popular method in use. However, UV induced fluorescence emission is of interest since derivatization of proteins with dansyl chloride, fluorescamine, o-phthaldialdehyde or coumarin moieties, is used to increase sensitivity.
CE is more versatile than gel electrophesis and it can be used to analyse proteins, nucleic acids, peptides, carbohydrates, anions, cations, vitamins, organic acids, amino acids, pesticides, even whole cells and viruses.

Table 5 Horizontal SCE-flat bed electrophoresis units

UniPhor type	L-280/165	L-280/80/2	L-165/260	XL -285/100/2
Product No.	Z64,071-9	Z64,072-7	Z64,074-3	Z64,075-1
Max. voltage	3,000 V	3,000 V	1,500 V	3,000 V
Max. current (mA)	25 mA	25 mA	50 mA	25 mA
Max. gel size	165 x 280 mm	165×280 mm	280×165 mm	200×290 mm
Min. electrode distance	4 cm	2 cm	8 cm	4 cm
Max. electrode distance	15 cm	8 cm	26 cm	20 cm
Overall dimensions (l x w x h)	29×33.5×7.5 cm	29×33.5×7.5 cm	29×33.5×7.5 cm	36,5×33×8,5 cm
Weight	3.5 kg	3.5 kg	3.5 kg	4 kg

Special Quality Grade for Ampholytes

As for other IEF applications ampholytes play a crucial role. Due to the fact that detection in CE is mostly based on UV, ampholytes for capillary IEF need to be completely transparent for UV. Thus we a range of ampholytes which is specifically specified and tested for absence of UV absorption.

PI-Markers

In order to ensure the high performance of analysis, standards of pI (pI-markers) are needed. In addition to classical protein based standards, low molecular compounds were developed and successfully examined in capillary IEF and IEF-gel electrophoresis. These markers are fluorescent, but can also be detected by UV-absorption at 280 nm (20°C). The maximum absorbances of the individual markers are between 308 and 350 nm. For fluorescence detection, an excitation wavelength of 310 nm (individual excitation maxim: 310 to 400 nm) is suggested; the emission maximum of the individual markers lies between 410 and 500 nm.

Table 7:
Fluorescent IEF-Markers and protein markers Stock solutions are suggested to be deluted 1:100

Cat. No.	Description	pI	Fluorescence		
			Em_{max} [nm]	Exc. [nm]	Buffer with pH = pI
35096 74169	solid solution	2.1	430	340	50 mM citrate, 50 mM KCl
17952 72172	solid solution	3.0	440	360	0.1 M citrate
17953 40677	solid solution	3.5	415	318	0.1 M citrate
17954 89827	solid solution	4.0	415	310	0.1 M citrate
17955 89149	solid solution	4.5	424	336	0.1 M citrate
17956 89478	solid solution	5.1	415	330	0.1 M citrate
17957 77866	solid solution	5.5	412	325	0.1 M citrate
17958 73938	solid solution	6.2	500	394	0.1 M phosphate
17959 73376	solid solution	6.6	500	396	0.1 M phosphate
17961 89508	solid solution	6.8	418	338	0.1 M phosphate
17962 89951	solid solution	7.2	500	387	0.1 M phosphate
17963 89952	solid solution	7.6	495	385	0.1 M tris
17964 75734	solid solution	8.1	420	340	0.1 M tris
17966 89357	solid solution	8.7	500	390	0.1 M tris
17967 90699	solid solution	9.0	495	385	0.1 M tris
46276 89268	solid solution	9.5	415	325	0.1 M carbonate
17968 77672	solid solution	10.3	495	388	0.1 M carbonate
17951	solution of marker-mix	4.0–9.0			
56730	mixture of 7 proteins, lyophilized	3.6–6.6			
56733	mixture of 8 proteins, lyophilzied	3.6–9.3			

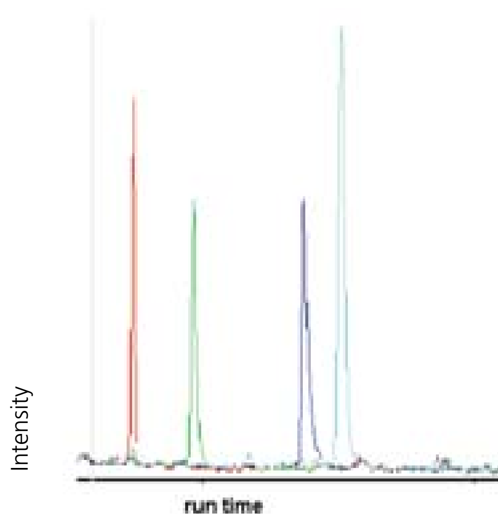


Figure 8:
Example for CE-IEF with fluorescent pI-markers; pI-marker: 8.7 (peak 1), 7.6 (peak 2), 6.6 (peak 3), 6.2 (peak 4)

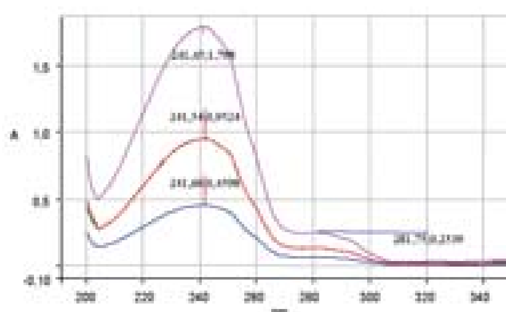


Figure 9:
The UV spectrum of the fluorescent IEF-marker pI 7.6 (three different concentrations)

Preparative Isoelectric Focussing

As IEF is based on non-denaturing conditions, it is well suited for purification of proteins, which will be obtained in active form and natural shape. An important application is prefractionation of proteins before loading onto pI-strips. Prefractionation is especially useful to circumvent problems of different abundance. Low abundance proteins may be separated from others and preconcentrated, so that they can be resolved and visualized in 2D-electrophoresis. Thus the combination of prefractionation step with 2D-electrophoresis may heavily extend the number of proteins which can be separated, detected and characterized. Preparative IEF has been performed with large and thick gels, but through the last few years several new formats of instruments for preparative focusing have been developed. For those, preparation is based on liquid phase electrofocusing within a chamber that in some cases is partitioned by membranes. For liquid phase electrofocusing typically free carrier ampholytes are used (Table 1 and 2)

Reference

M. Horka, Th. Willimann, M. Blum, P. Nording, Z.Friedl, K. Slais, Capillary isoelectric focusing with UV-induced fluorescence detection, J. of Chromatography A, 916 65-71 (2001)

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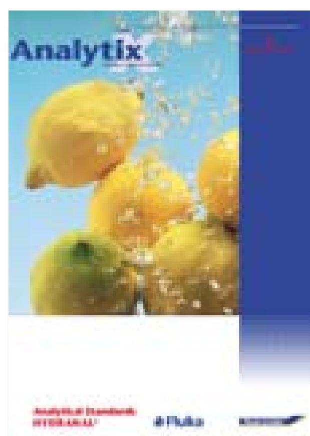
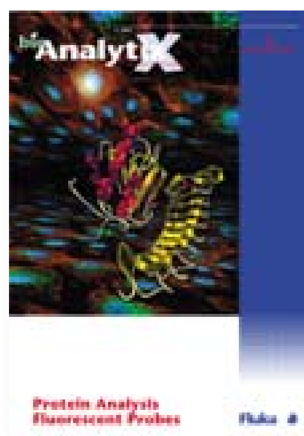
Analytical Standards HYDRANAL®





Protein Analysis Fluorescent Probes

Derivatization Reagents

Microbiology

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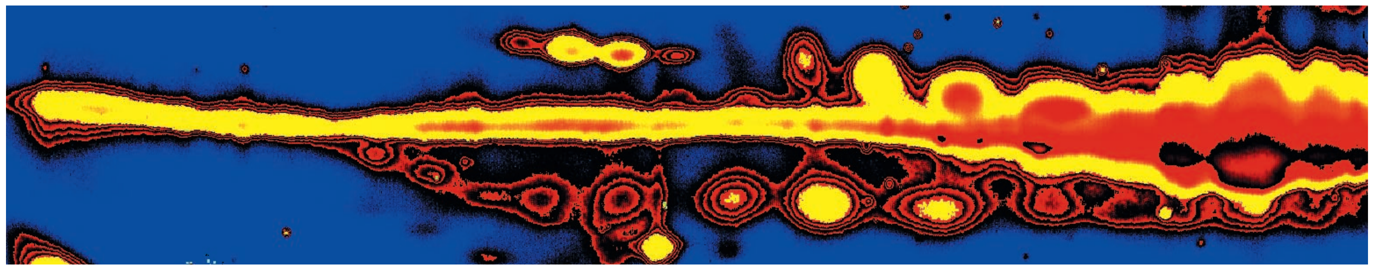
FRH 5.2003/30



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39878 Ampholyte high resolution pH 3-10 **one free sample of 1 ml**

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