Improved **Immobiline** DryStrip gels and IPG Buffer

2-D ELECTROPHORESIS

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Immobiline™ DryStrip gels (IPG strips) are used for isoelectric focusing (IEF; Fig 1), run as the first dimension of 2-D electrophoresis or as a separate application. IPG strips contain a preformed pH gradient immobilized in precast polyacrylamide gels on a plastic backing. A comprehensive range of overlapping IPG strips covering narrow, medium, and wide pH ranges are available in various strip lengths, and the strips are individually packaged and bar coded for convenient handling and sample tracking. When used for 2-D DIGE applications, the gels give accurate and reliable 2-D results.

Key features:

- Immobiline DryStrip gels have been improved on several levels, through new process parameters, to produce first and second dimension gels of overall higher quality
- Improved Immobiline DryStrip gels provide increased stability, reproducibility, and protein spot definition
- Better spot definition improves spot quantitation in data analysis
- Stable and accurate immobilized pH gradients and precise lengths ensure high reproducibility and reliable gel comparisons
- Wide selection of IPG strips, pH gradients, lengths, and buffers for greater versatility
- Immobiline DryStrip gels with matching IPG Buffer gives a high sample loading capacity and improves resolution

Improved Immobiline DryStrip gels provide increased spot definition

Immobiline DryStrip gels have been improved to increase stability, reproducibility, and protein spot definition. Figure 2A shows a case of skewed bands in the first dimension giving rise to elongated spots in the second dimension. In Figure 2B, the improved stability of the pH gradient gives straight bands in the first dimension and better spot definition and spot focusing in the second dimension. With improved Immobiline DryStrip gels, increased spot definition leads to accurate matching and analysis.



Fig 1. Using Immobiline DryStrip gels with IPG Buffer maximizes resolution, loading capacity, and reproducibility of first dimension IEF, which greatly improves the quality of your 2-D electrophoresis results. Each IPG strip is individually packed for extended durability.

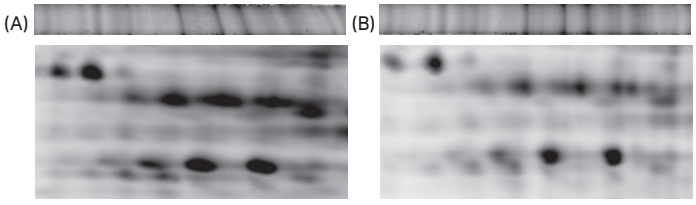
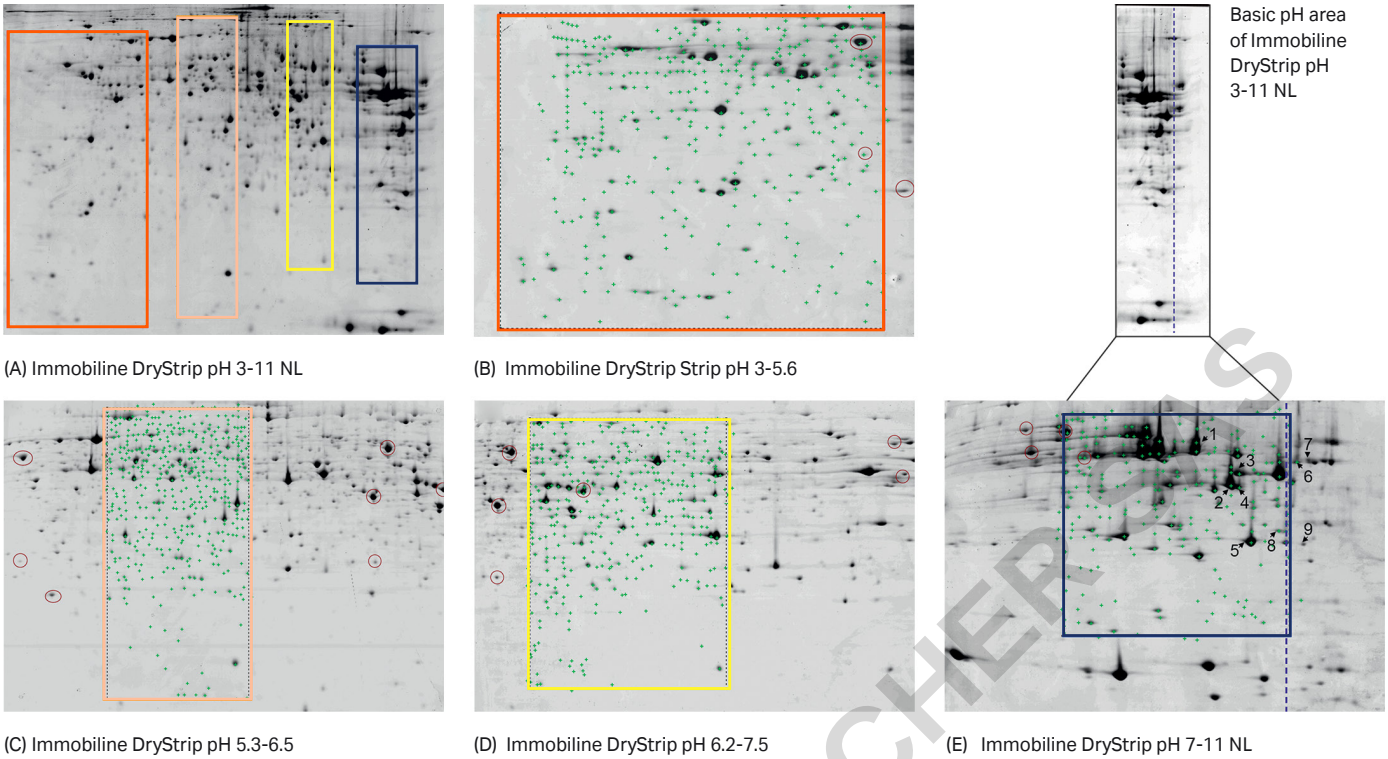


Fig 2. Immobiline DryStrip pH 4-7 run according to standard procedures. (A) Spots are elongated in the second dimension due to skewed bands in the first dimension. (B) Improved Immobiline DryStrip gels provide a more stable pH gradient and better spot definition in the second dimension.



(A) Immobiline DryStrip pH 3-11 NL

(B) Immobiline DryStrip Strip pH 3-5.6

(C) Immobiline DryStrip pH 5.3-6.5

(D) Immobiline DryStrip pH 6.2-7.5

(E) Immobiline DryStrip pH 7-11 NL

Fig 3. 2-D gel images using (A) wide range Immobiline DryStrip pH 3-11NL or (B)-(E) four overlapping narrow range IPG strips. Immobiline DryStrip pH 7-11 NL is able to resolve a number of spots that were lost in the basic area of Immobiline DryStrip pH 3-11 NL.

Higher resolution using narrow range Immobiline DryStrip gels

To maximize resolution, four overlapping narrow range IPG strips were used and the spot maps were analyzed using ImageMaster™ 2D Platinum. The analysis was compared to data obtained using the wide range Immobiline DryStrip pH 3-11 NL, and the analyses revealed a 200% increase of detected protein spots. Figure 3 shows the gel images from the narrow and wide range IPG strips. Figure 3E shows that Immobiline DryStrip pH 7-11 NL was able to resolve several spots that were lost in the basic area of Immobiline DryStrip pH 3-11 NL.

Improved IPG Buffer pH 3-10 and pH 3-10 NL increases resolution

IPG Buffer pH 3-10 and pH 3-10 NL have been improved with a new formula. The improved buffer formula was compared with the old formula based on the resulting 2-D spot maps. The quality of the gels was evaluated both by a panel of six experienced 2-D users and by software analysis using ImageMaster 2D Platinum. The panel evaluated several triplicate sets of 2-D spot maps using either improved IPG Buffer or previous IPG Buffer formulations, and based their evaluation on the numbers of spots, intensity, and focusing of the spots. Using ImageMaster 2D Platinum, the 2-D maps were evaluated based on spot intensity, spot matching, and spot numbers. The basic pH area of two 2-D spot maps is shown in Figure 4, while Figure 5 shows the complete 2-D spot maps. Both investigation methods demonstrated that the improved buffers gave additional spots in the high molecular weight area and basic pH area of the gel, as well as spots with increased intensities.

By increasing the intensity of low intensity spots, the probability of detecting low abundant proteins is increased. Note that the improved formula for IPG Buffer pH 3-10 and pH 3-10 NL gives rise to a higher background in the basic pH area of the gel when using silver or Coomassie™ staining. This higher background can be avoided by prolonging the fixation time to 3 hours or up to overnight if needed.

Sample labeling:	Lysed *E.coli* cells (50 µg) labeled with CyDye™ DIGE Fluor, Cy™5 minimal dye
Sample buffer:	8 M urea, 4% CHAPS, 40 mM DTT, and 2% IPG Buffer pH 3-10 NL.
First dimension IEF:	Immobiline DryStrip gels (pH 3-10 NL; 18 cm) were rehydrated overnight at room temperature with Destreak Rehydration Solution and 0.5% IPG Buffer pH 3-10 NL. The sample was applied by anodic cup loading and focused for 24 kVh.
Second dimension:	Run on Ettan™ DALT*twelve* electrophoresis system using 12.5% labcast Laemmli gels.

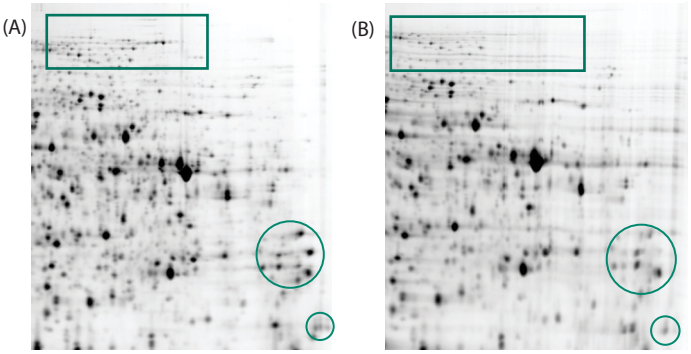


Fig 4. The basic area of 2-D protein spot maps using Immobiline DryStrip pH 3-10 NL (18 cm) together with (A) improved IPG Buffer pH 3-10 NL or (B) the previous recipe for IPG Buffer pH 3-10 NL. The improved IPG Buffer formula results in additional spots, and some of the differences are indicated in the figure.

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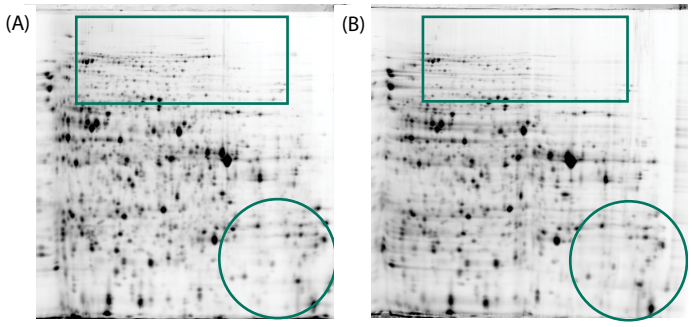


Fig 5. 2-D protein spot maps using Immobiline DryStrip pH 3-10 NL (18 cm) together with (A) improved IPG Buffer pH 3-10 NL or (B) the previous IPG Buffer pH 3-10 NL formulation. Experimental conditions were identical to the conditions in Figure 4.

Choosing IPG strip and buffer

A comprehensive range of overlapping IPG strips covering narrow (~1 pH unit), medium (3-5 pH units), and wide (~8 pH units) pH ranges are available in five different strip lengths (7, 11, 13, 18, and 24 cm). To simplify gel use and record keeping, each strip is labeled with the pH interval, batch number, individual number, and bar code for use with a reader. Figure 6 shows an overview of the IPG strips available along with the matching IPG Buffers. The combination of IPG strip and IPG Buffer of matching pH interval improves the conductivity distribution across the pH gradient during IEF. The technical specifications for Immobiline DryStrip gels and IPG Buffers are shown in Table 1.

To gain a broad overview of total protein distribution, select wide range IPG strips such as Immobiline DryStrip pH 3–11 NL. Choose shorter strips, up to 13 cm, for fast, cost-effective screening, for a quick overview, or when only the most abundant proteins are of interest (as in prefractionated protein samples). The shortest IPG strips give the fastest results, but their sample load is limited. For more detailed studies, choose medium range IPG strips. To obtain higher resolution, narrow range IPG strips may be used. Use the longer 18 and 24 cm strips for maximal resolution and loading capacity. Longer strips allow detection of more spots and make it easier to select and identify the proteins in the spots, but require longer focusing times. For a more detailed insight into protein distribution, combine medium range Immobiline DryStrip pH 3–7 (or pH 4–7) and pH 6–11 (alternatively, pH 6–9 or pH 7-11). This combination is also useful for preparative applications. Medium range IPG strips in the 24 cm long format deliver both high resolution and high protein loading capacity, both of which improve protein identification and characterization later in the workflow. Deep Purple, Coomassie, or silver stained spots can be picked from the gel and identified by mass spectrometry.

Table 1. Technical specifications for Immobiline DryStrip gels and IPG Buffer

Immobiline DryStrip	
Gel dimensions	70 × 3 × 0.5 mm, 110 × 3 × 0.5 mm, 130 × 3 × 0.5 mm, 180 × 3 × 0.5 mm, 240 × 3 × 0.5 mm
Gel matrix	Polyacrylamide T = 4%, C = 3%
Gel backing	Polyester film
Storage	-20°C
Shelf life	18 months
IPG Buffer	
Content	Specialized carrier ampholytes in aqueous solution
Volume	1 mL
Storage	4°C to 8°C
Shelf life	36 months

	IPG Strips					IPG Buffers							
	Strip length					pH range							
	24 cm	18 cm	13 cm	11 cm	7cm	3.5-5.0	5.5-6.7	4-7	6-11	7-11 NL	3-10 NL	3-10	3-11 NL
Narrow													
3.5-4.5	×					•							
5.3-6.5	×	×	×	×	×		•						
6.2-7.5	×	×	×	×	×				•				
Medium													
3-5.6 NL	×	×	×	×	×	•							
3-7 NL	×							•					
4-7	×	×	×	×	×			•					
6-9	×	×							•				
6-11		×	×	×	×				•				
7-11 NL	×	×	×	×	×					•			
Wdide													
3-10	×	×	×	×	×							•	
3-11 NL	×	×	×	×	×								•
3-10 NL	×	×	×		×						•		

Fig 6. A comprehensive range of overlapping IPG strips covering narrow, medium, and wide pH ranges are available in several different strip lengths. Using IPG strips together with matching IPG Buffer improves the conductivity distribution across the pH gradient during IEF.

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IPGbox

Immobiline DryStrip gel packs are shipped dry for stability. IPGbox provides a convenient method for rehydrating up to twelve precast IPG strips (7 to 24 cm) at a time. Individual slots in the Reswell Trays allow rehydration of individual IPG strips in a minimum volume of solution, and no oil is required. IPGbox can accommodate all IPG strip sizes including 7, 11, 13, 18, and 24 cm (Fig 7). IPGbox is supplied with a complete set of accessories to rehydrate 10 full rounds of 12 strips each. To avoid redox-related streaking, IPG strips should be rehydrated with DeStreak Rehydration Solution.



Fig 7. IPGbox is specially designed for rehydrating Immobiline DryStrip gels in a minimum volume of solution.

Synchronized solutions for better 2-D electrophoresis — improved 2-D DIGE

2-D electrophoresis is a powerful technology for comparing complex protein mixtures from biological samples in proteomics research. With the aim of simplifying the process and improving quantitation, Cytiva has made significant improvements across the entire workflow, from sample prep and separation to data analysis. 2-D DIGE (Difference Gel Electrophoresis) allows you to analyze differences in low abundance proteins with unparalleled statistical confidence. Many of our 2-D electrophoresis products improve data quality from traditional 2-D experiments as well, and the products can eventually be integrated into a complete solution to maximally improve your 2-D results.



Sample Preparation	Protein Labeling	Protein Separation	Image Acquisition	Image Analysis	Validation
2-D Protein Extraction Buffer	DIGE Trial Pack CyDye DIGE Fluor 2 nmol kit CyDye DIGE 400 nmol sat dye CyDye DIGE repackaging	IPGbox Immobiline DryStrip gels IPG Buffer DIGE Gel and DIGE Buffer Kit	Ettan DIGE Imager Typhoon™ Variable Mode Imager	DeCyder™ 2-D software v7.0	ECL Plex™ ImageQuant™ TL 7.0 with ImageQuant TL SecurITy

For more information, visit **cytiva.com/2DE**

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Ordering information

Product	Quantity	Code No
IPG Buffer pH 3.5-5.0	1 mL	17-6002-02
IPG Buffer pH 5.5-6.7	1 mL	17-6002-06
IPG Buffer pH 4-7	1 mL	17-6000-86
IPG Buffer pH 6-11	1 mL	17-6001-78
IPG Buffer pH 7-11 NL	1 mL	17-6004-39
IPG Buffer pH 3-10	1 mL	17-6000-87
IPG Buffer pH 3-10 NL	1 mL	17-6000-88
IPG Buffer pH 3-11 NL	1 mL	17-6004-40

Related literature	Code No
2-D Electrophoresis: Principles and methods, Handbook	80-6429-60
Ettan DIGE System User manual	18-1173-17

Related products	Quantity	Code No
IPGbox	1 IPGbox + 1 IPGbox Kit	28-9334-65
IPGbox Kit	10 Reswell Trays + 1 IPGbox Insert	28-9334-92
DeStreak Rehydration Solution	5 × 3 mL	17-6003-19
DeStreak Reagent	1 mL	17-6003-18
Ettan IPGphor™ 3 Isoelectric Focusing Unit	1	11-0033-64
Ettan IPGphor Manifold, Complete	1	80-6498-38
Ettan IPGphor Manifold, Light Complete	1	11-0026-88

Immobiline DryStrip gels[1]	Code No		
Narrow range	**pH 3.5-4.5**	**pH 5.3-6.5**	**pH 6.2-7.5**
24 cm	17-6002-38	17-6003-62	17-6003-67
18 cm	–	17-6003-61	17-6003-66
13 cm	–	17-6003-60	17-6003-65
11 cm	–	17-6003-59	17-6003-64
7 cm	–	17-6003-58	17-6003-63

Medium range	pH 3-5.6 NL	pH 3-7 NL	pH 4-7	pH 6-9	pH 6-11	pH 7-11 NL
24 cm	17-6003-57	17-6002-43	17-6002-46	17-6002-47	–	17-6003-72
18 cm	17-6003-56	–	17-1233-01	17-6001-88	17-6001-97	17-6003-71
13 cm	17-6003-55	–	17-6001-13	–	17-6001-96	17-6003-70
11 cm	17-6003-54	–	18-1016-60	–	17-6001-95	17-6003-69
7 cm	17-6003-53	–	17-6001-10	–	17-6001-94	17-6003-68

Wide range	pH 3-10	pH 3-10 NL	pH 3-11 NL
24 cm	17-6002-44	17-6002-45	17-6003-77
18 cm	17-1234-01	17-1235-01	17-6003-76
13 cm	17-6001-14	17-6001-15	17-6003-75
11 cm	18-1016-61	–	17-6003-74
7 cm	17-6001-11	17-6001-12	17-6003-73

[1] Pack size is 12 strips/pack

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DIGE Gel and DIGE Buffer Kit: The buffer system in this gel and buffer kit is covered by patent application WO9616724 granted in US, EP and JP.

This version of ImageMaster has been developed by the Swiss Institute of Bioinformatics in collaboration with GeneBio and Cytiva.

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