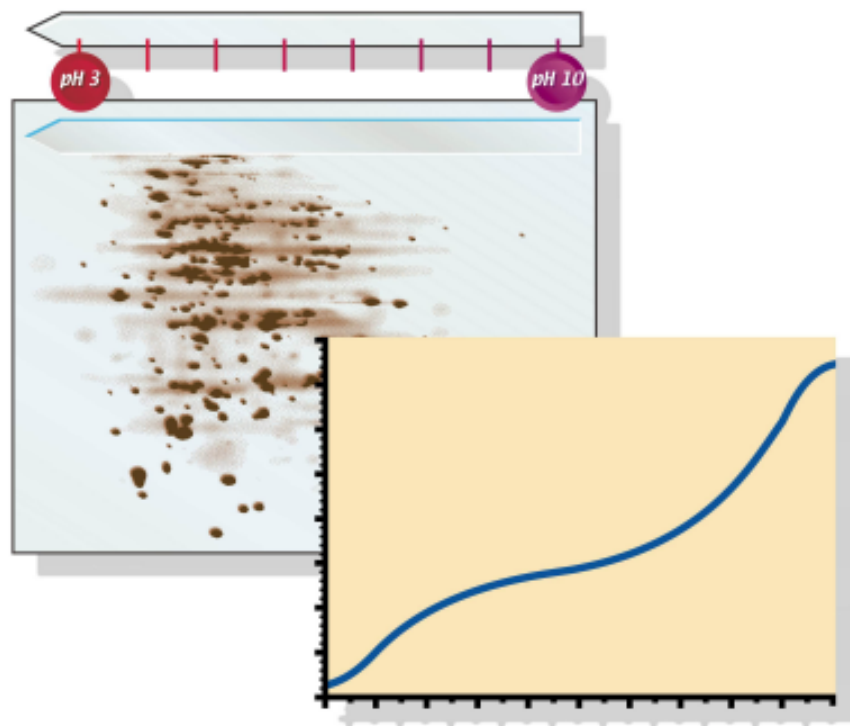


# Immobiline DryStrip Visualization of pH gradients

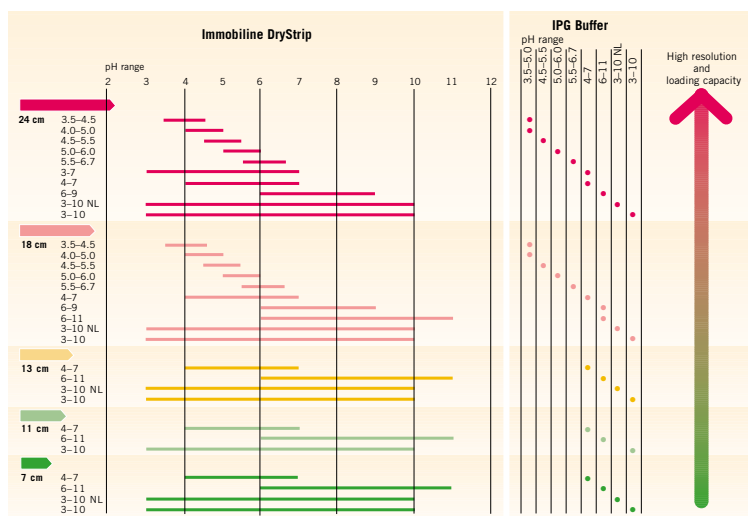


# Immobiline DryStrip as the first di

## Setting the standard

Products from Amersham Pharmacia Biotech set the standard for successful, highly reproducible 2-D electrophoresis, using Immobiline™ DryStrip immobilised pH gradient gels in IPGphor™ or Multiphor™ II for the first dimension and SDS gels on Hoefer™ vertical systems or Multiphor II flatbed system for the second dimension separation.

Immobiline DryStrip gels are easy to handle and highly reproducible, providing a wide selection of pH ranges and strip lengths (shown in the table below) to simplify the development of clear strategies for the analysis of complex cell extracts. The excellent first dimension separation ensures that spots in the second dimension are well separated, even with high protein loads.



## Choosing strip length

Immobiline DryStrip gels provide overlapping coverage from pH 3 to pH 10 and are available in five lengths: 7, 11, 13, 18 and 24 cm.

Choose shorter strips for fast, cost-effective screening purposes (for a quick overview) or when only the most abundant proteins are of interest (as in pre-fractionated protein complexes). The shortest IPG strips give the fastest results, but the sample load is limited.

Use longer strips for maximal resolution and loading capacity. Longer strips allow the detection of more spots and facilitate the selection and identification of proteins in the spots. But remember that longer focusing times will be needed in both the first dimension and the second dimension separation. Table 1 gives an example of the inter-relationship between these parameters.

Table 1. Typical operating parameters for Immobiline DryStrip pH 4-7 gels with *E. coli* extract. Protein spots were detected by silver staining. A similar relationship between number of spots detected and gel length is observed with other pH intervals and staining methods if the sample load is changed accordingly.

Parameters	7 cm	11 cm	13 cm	18 cm	24 cm
Time 1st dimension (h)	2	4	4	6	10
Time 2nd dimension (h)	1.5	2	3	5	5.5
Sample (µg proteins)	10	25	30	55	90
Typical number of spots detected	350	500	600	850	1000



# Dimension in 2D electrophoresis:

## Guide lines to choosing gel length, pH gradient and estimation of pI values.

### Choosing pH gradients

Use a pH interval of 3–10 for an overview of total protein distribution. With a linear gradient pH 3–10 strip the estimation of a protein's isoelectric point (pI) is relatively easy.

For increased resolution between pH 5 and 7, use a non-linear gradient pH 3–10 strip to distribute the proteins more evenly over the gel. This is particularly helpful when analysing cell extracts as, when run on a linear gradient, these often display a crowded area of spots between pH 4.5 and 6.0 and relatively few proteins at the basic end.

Combine pH 4–7 and pH 6–11 (or pH 4–7 and pH 6–9) to obtain a more detailed overview of the protein distribution. This combination is used also for preparative applications.

Follow this analysis by using narrow pH range (1 pH unit) Immobiline DryStrip gels to study, in more detail or with greater loads, the proteins in the regions of interest. These narrow pH range (1 pH unit) 24-cm IPG strips deliver the highest resolution and protein loading capacity available. Coomassie™ stained spots are cut out and identified by MALDI/TOF mass spectrometry.

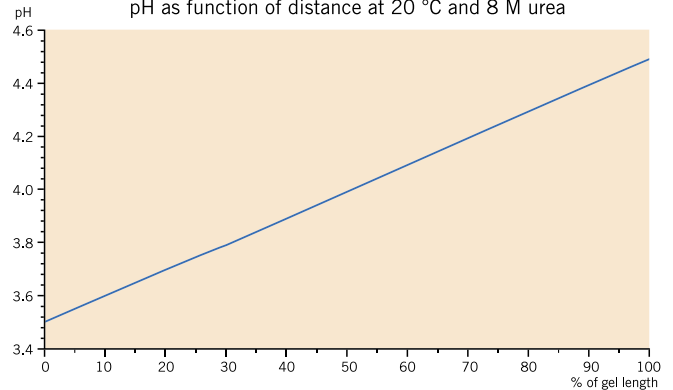
### Precision and reproducibility of pH gradients

A high level of performance with guaranteed reproducibility is essential for successful 2-D electrophoresis. The statistical analysis below based on 80 batches and over 7 years of production clearly demonstrate the excellent accuracy achievable on Immobiline DryStrip gels. Batch-to-batch reproducibility of the pH gradient is extremely high, with standard deviation (s) of less than  $\leq 1\%$  for the position of specific pH values along the gradient (as defined by the relative position [Rp] of standard proteins and shown in Table 2).

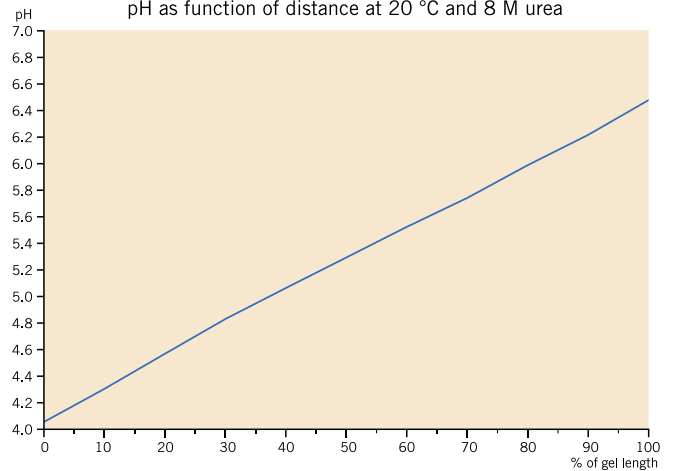
Table 2. Statistical analysis of average Rp values of standard proteins of Immobiline DryStrip pH 3-10NL, 18 cm (17-1235-01) based on 80 batches and over 7 years of production.

Sdt no.	1	2	3	4	5	Av
Av Rp	0.24	0.45	0.69	0.78	0.83	–
$\sigma$	0.009	0.007	0.007	0.008	0.010	0.008

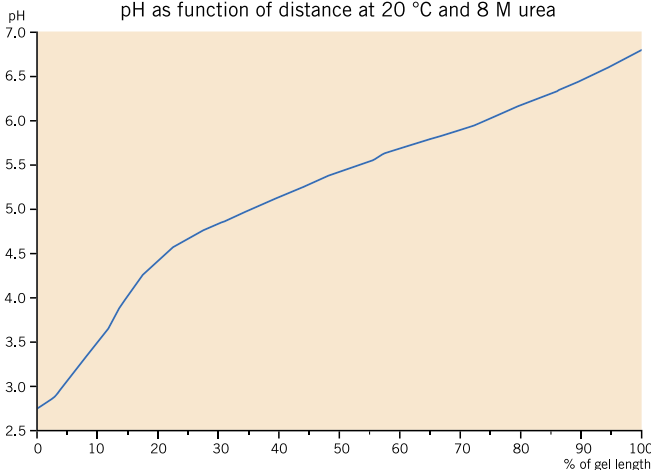
Immobiline DryStrip pH 3.5 - 4.5, 18 and 24 cm  
pH as function of distance at 20 °C and 8 M urea



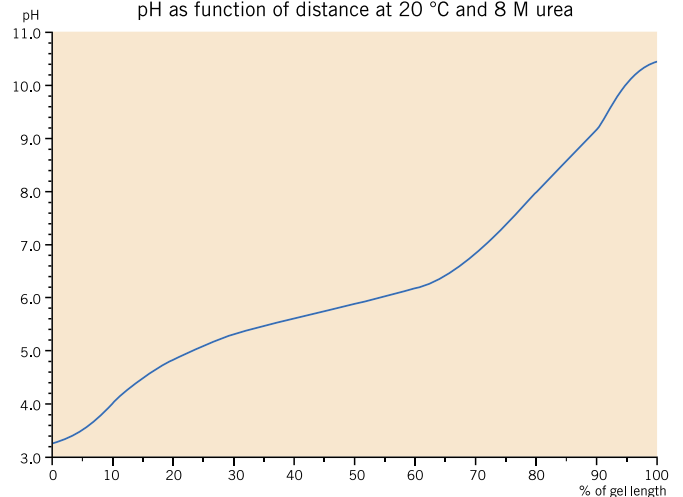
Immobiline DryStrip pH 4 - 7\*, 7, 11, 13, 18 and 24 cm  
pH as function of distance at 20 °C and 8 M urea

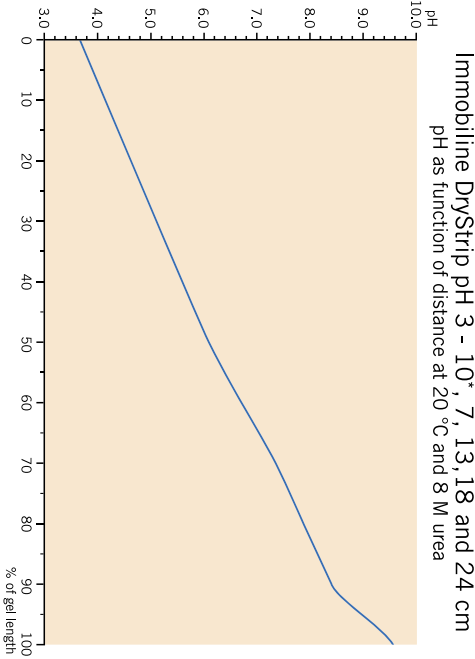
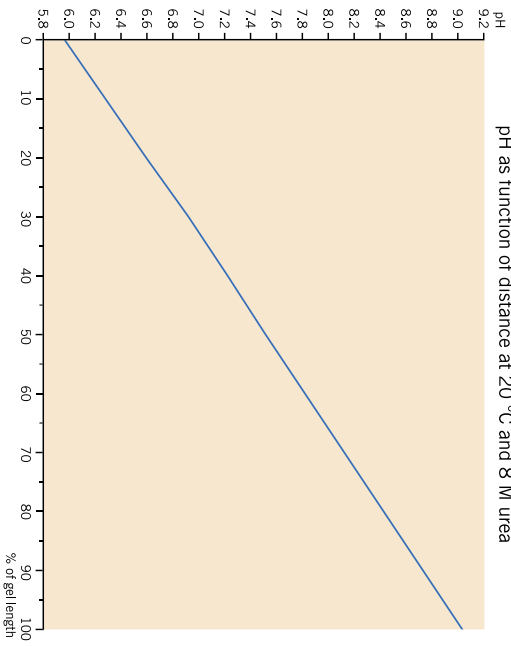
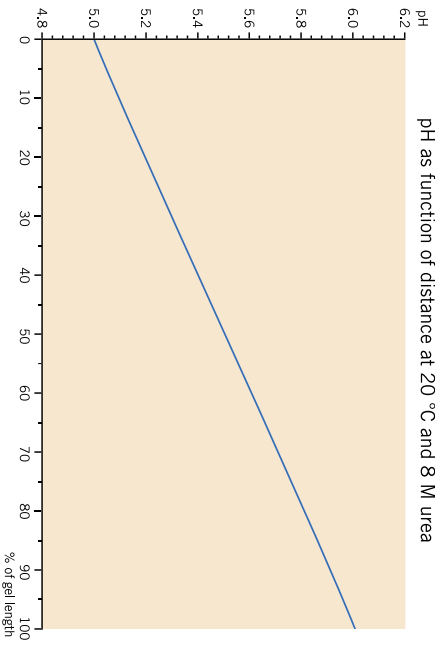
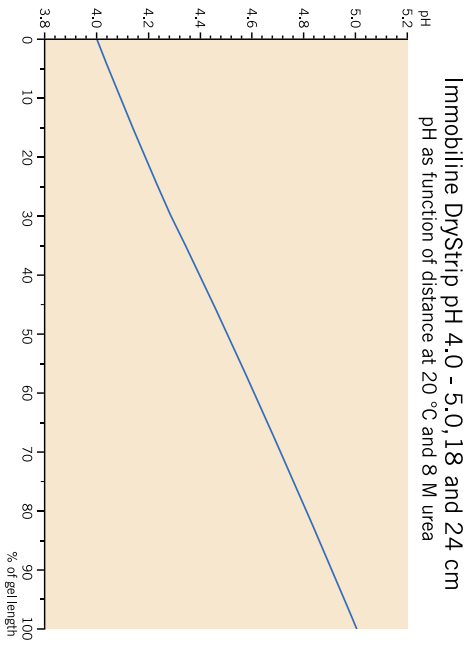


Immobiline DryStrip pH 3 - 7 NL, 24 cm  
pH as function of distance at 20 °C and 8 M urea

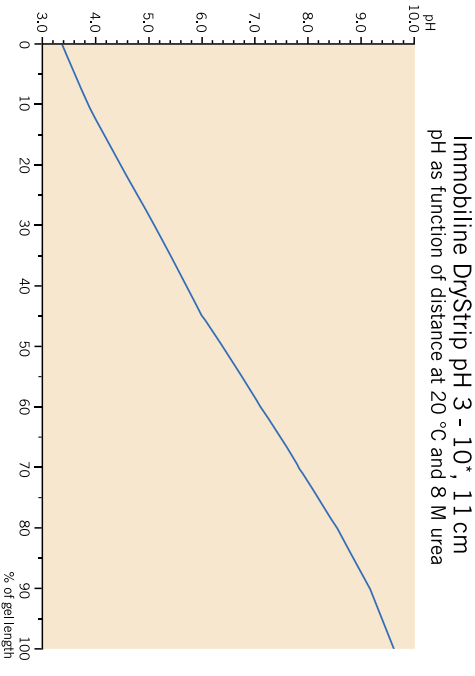
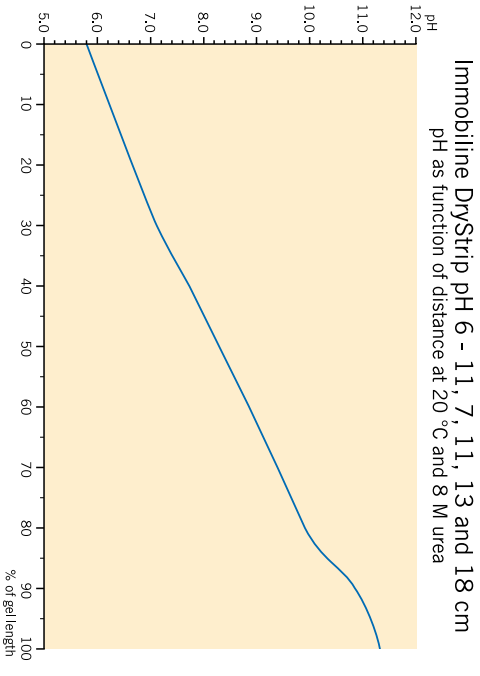
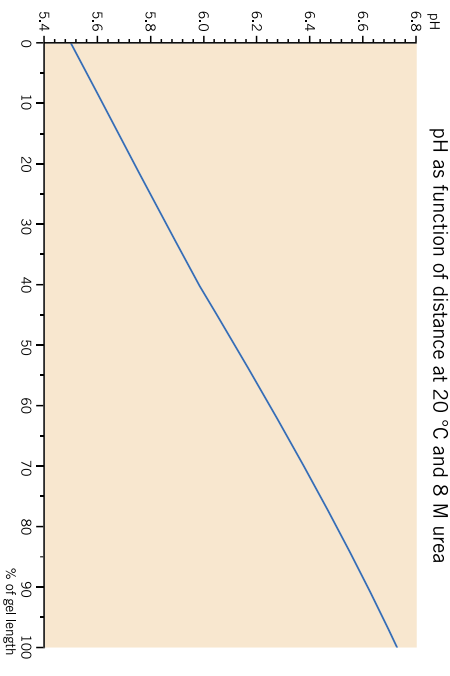
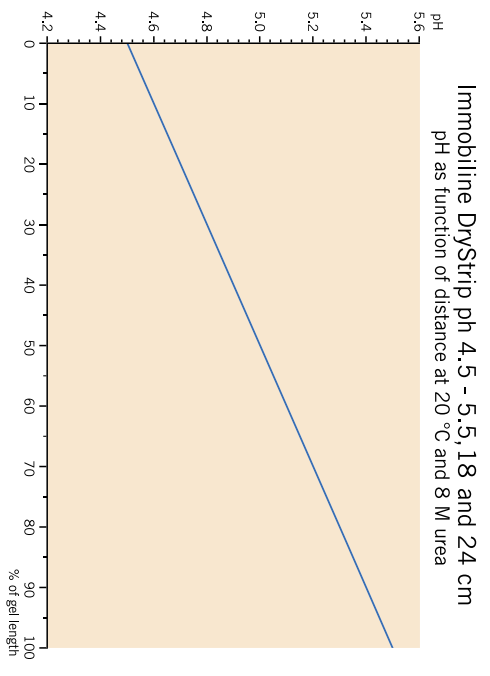


Immobiline DryStrip pH 3 - 10NL, 7, 13, 18 and 24 cm  
pH as function of distance at 20 °C and 8 M urea





\*These gels were originally developed for native isoelectric focusing in pure water and were named accordingly. Using the same gels in the presence of urea means that the actual pH range does not correspond exactly to the product name.



# dimension in 2D electrophoresis

## pH gradient profiles

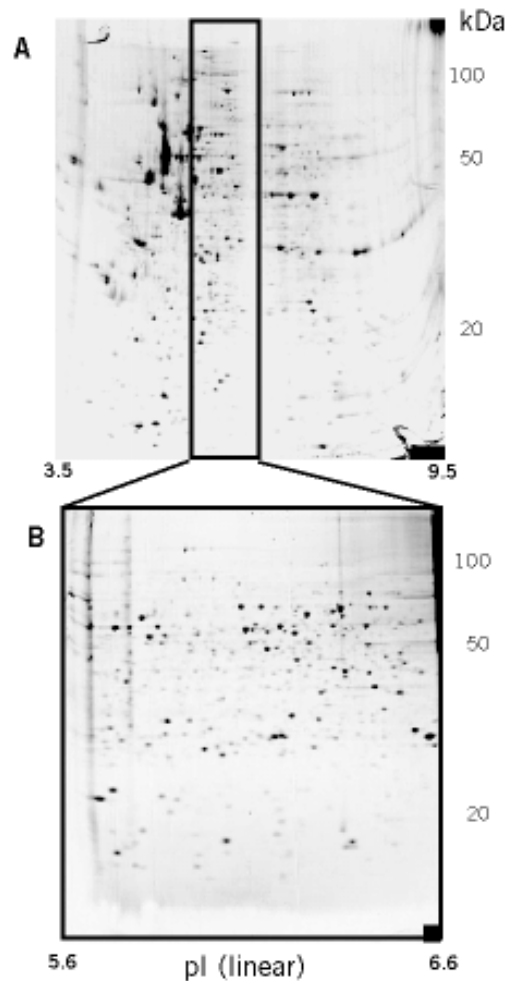
The pH gradients are the calculated\* target pH gradient profiles, run at 20 °C in 8 M urea, 19 mM DTT and with a co-ordinated IPG buffer.

\*The shape of a pH gradient is determined by reference to proteins of known pI, run on the same gel, and calculated from the concentration and pKs of the acids and bases used to form the gradient. When calculating the gradient shape, the solvent and temperature must be taken into account. The pK values for weak acids and bases are dependent on the solvent used, particularly the concentrations of urea. Temperature affects the pKs of each buffer species differently.

## Estimating pI after 2-D electrophoresis

The reliability of the first dimension separation is so high that the pI of a protein can be estimated by relating the position of the protein in the second dimension gel to its original position in the Immobiline DryStrip gel.

Determine the first dimension position by measuring the length of the Immobiline DryStrip gel, the position of the strip on the second dimension gel and, for gels not attached to backing, correct for shrinkage or swelling of the gel during staining. Plot the spot position (as a per cent of gel length) versus pH and read off the pI from the graph of the pH gradient. Larger graphs and more detailed information on this procedure can be found at [http:// proteomics.apbiotech.com](http://proteomics.apbiotech.com)



A 2-D map of Hek proteins analysed by ImageMaster 2D Elite software. (A) First dimension pH 3-10 gradient strip. (B) First dimension narrow pH 5.5-6.7 gradient strip. (Results courtesy of Dr Hanno Langen, F. Hoffmann-La Roche AG, Basel, Switzerland.)

For more details see article "Separation of Proteins from Human Embryonic Kidney Cells on Narrow Range pH Strips". H. Langen and D. Röder. Life Science News 3, (1999).

# Technical data and ordering information

## Technical data

### Immobiline DryStrip

Gel dimensions: 235 x 3 x 0.5 mm  
 180 x 3 x 0.5 mm  
 130 x 3 x 0.5 mm  
 110 x 3 x 0.5 mm  
 70 x 3 x 0.5 mm

Gel matrix: Polyacrylamide T=4%, C=3%  
 Gel backing: Polyester film  
 Shelf-life: 18 months  
 Storage: -20 °C

## Technical data

### IPG Buffer

Content: Specialized carrier ampholytes  
 in aqueous solution  
 Volume: 1 ml  
 Storage: +4 °C to +8 °C

## Ordering information

### Sample preparation

IPG Buffer	Code No.	IPG Buffer pH 3.5-5.0	Code No.
IPG Buffer pH 4-7, 1 ml	17-6000-86	IPG Buffer pH 4.5-5.5	17-6002-04
IPG Buffer pH 6-11, 1 ml	17-6001-78	IPG Buffer pH 5.0-6.0	17-6002-05
IPG Buffer pH 3-10, 1 ml	17-6000-87	IPG Buffer pH 5.5-6.7	17-6002-06
IPG Buffer pH 3-10 NL, 1 ml	17-6000-88		

Ampholyte concentrate in aqueous solution, 1.0 mL

### First dimension IEF

#### Immobiline DryStrip

Dry polyacrylamide gels (0.5 mm, T=4%, C=3%, after rehydration) cast on plastic backing. 12/pk.

	Narrow	24 cm	18 cm	13 cm	11 cm	7 cm
pH 3.5-4.5	17-6002-38		17-6001-83			
pH 4.0-5.0	17-6002-39		17-6001-84			
pH 4.5-5.5	17-6002-40		17-6001-85			
pH 5.0-6.0	17-6002-41		17-6001-86			
pH 5.5-6.7	17-6002-42		17-6001-87			
Medium						
pH 3-7 NL	17-6002-43					
pH 4-7	17-6002-46	17-1233-01	17-6001-13	18-1016-60	17-6001-10	
pH 6-9	17-6002-47	17-6001-88				
pH 6-11		17-6001-97	17-6001-96	17-6001-95	17-6001-94	
Wide						
pH 3-10	17-6002-44	17-1234-01	17-6001-14	18-1016-61	17-6001-11	
pH 3-10 NL	17-6002-45	17-1235-01	17-6001-15	N/A	17-6001-12	

<http://proteomics.apbiotech.com>

### Asia Pacific

Amersham Pharmacia Biotech Asia Pacific Ltd  
 Tel: +852 2811 8693  
 Fax: +852 2811 5251

### Australasia

Amersham Pharmacia Biotech Australia Pty Ltd  
 Tel: +61 2 9894 5152  
 Fax: +61 2 9899 7511

### Austria

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 01 576 0616 24  
 Fax: 01 576 0616 27

### Belgium

Amersham Pharmacia Biotech Benelux  
 Tel: 0800 73888  
 Fax: 03 272 1637

### Canada

Amersham Pharmacia Biotech, Inc.  
 Tel: 1 800 463 5800  
 Fax: 1 800 567 1008

### Central, East & South East Europe

Amersham Pharmacia Biotech Export GmbH  
 Tel: +43 1 982 3826  
 Fax: +43 1 985 8327

### Denmark

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 4516 2400  
 Fax: 4516 2424

### Finland

Amersham Pharmacia Biotech Europe GmbH  
 Tel: +358-(0)9-512 39 40  
 Fax: +358-(0)9-512 17 10

### France

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 0169 35 67 00  
 Fax: 0169 41 96 77

### Germany

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 0761 4903 402  
 Fax: 0761 4903 405

### Italy

Amersham Pharmacia Biotech Italia  
 Tel: 02 27322 1  
 Fax: 02 27302 212

### Japan

Amersham Pharmacia Biotech KK  
 Tel: 81 3 5331 9336  
 Fax: 81 3 5331 9370

### Latin America

Amersham Pharmacia Biotech do Brasil Ltda  
 Tel: +55 11 3667 5700  
 Fax: +55 11 3667 8799

### Middle East and Africa

Amersham Pharmacia Biotech Export GmbH  
 Tel: +30 (1) 96 00 687  
 Fax: +30 (1) 96 00 693

### Netherlands

Amersham Pharmacia Biotech Benelux  
 Tel: 0165 580 410  
 Fax: 0165 580 401

### Norway

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 23 18 58 00  
 Fax: 23 18 68 00

### Portugal

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 21 417 70 35  
 Fax: 21 417 31 84

### Russian Federation

ZAO Amersham Pharmacia Biotech  
 Tel: +7 (095) 232 0250  
 Fax: +7 (095) 230 6377

### South East Asia

Amersham Pharmacia Biotech AB  
 Tel: 60 3 724 2080  
 Fax: 60 3 724 2090

### South East Europe

Amersham Pharmacia Biotech GmbH  
 Tel: +43 (1) 982 3826  
 Fax: +43 (1) 982 8327

### Spain

Amersham Pharmacia Biotech Europe GmbH  
 Sucusal en España  
 Tel: 93 594 49 50  
 Fax: 93 594 49 55

### Sweden

Amersham Pharmacia Biotech Sverige  
 Tel: 018 612 19 00  
 Fax: 018 612 19 10

### Switzerland

Amersham Pharmacia Biotech Europe GmbH  
 Tel: 01 802 81 50  
 Fax: 01 802 81 51

### UK

Amersham Pharmacia Biotech UK Limited  
 Tel: 0800 616 928  
 Fax: 0800 616 927

### USA

Amersham Pharmacia Biotech Inc  
 Tel: +1 800 526 3593  
 Fax: 877 295 8102

Hoefler, Immobiline, IPGphor and Multiphor are trademarks of Amersham Pharmacia Biotech Limited or its subsidiaries. Amersham is a trademark of Nycomed Amersham plc. Pharmacia and Drop Design are trademarks of Pharmacia & Upjohn Inc. Coomassie is a trademark of ICI plc. Amersham Pharmacia Biotech AB Björkgatan 30, SE-751 84 Uppsala, Sweden. Amersham Pharmacia Biotech UK Limited Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, England. Amersham Pharmacia Biotech Inc 800 Centennial Avenue, PO Box 1327, Piscataway, NJ 08855 USA. Amersham Pharmacia Biotech Europe GmbH Munzinger Strasse 9, D-79111 Freiburg, Germany. Amersham Pharmacia Biotech K.K. Sanken Building, 3-25-1, Shinjuku-ku, Tokyo 169-0073, Japan. All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Pharmacia Biotech group which supplies them. A copy of these terms and conditions of sale is available on request.  
 © Amersham Pharmacia Biotech AB 2000 - All rights reserved.



amersham pharmacia biotech